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SEDIMENT-BASED INTRINSIC BIOREMEDIATION at ROBINS AIR FORCE BASE

Prepared for

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by

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Summary

Microbial activity in root-zone sediments from wetlands adjacent to the Robins AFB was investigated to determine the potential for attenuation of contaminants in surface-runoff and groundwater flows into the wetland system. The wetland system is downgradient from Robins AFB and will potentially receive inputs of contaminants of concern (COC) from landfill leachates, spills and subsurface contamination from past industrial operations.

The wetland sediments are highly-active biological media with respect to metabolism of natural-organic matter, readily-degradable organics and investigated COCs. Aerobic and anaerobic (methanogenic) activity were examined using oxygen-respiration, methane-production and microbial-count procedures. COCs were examined using benzene, chlorobenzene, 1,4-dichlorobenzene, trichloroethylene (TCE), acetone, and phenol as indicative compounds. In aerobic systems and without the use of acclimation techniques, the native microbial population of the wetland sediments were able to rapidly degrade benzene, chlorobenzene, 1,4-dichlorobenzene, acetone and phenol, as well as natural organic matter in the sediments and five easily-degraded compounds used in the study. TCE was not degraded by the sediments and TCE exhibited potential for inhibition of natural microbial activity. In anaerobic systems, acetone and phenol were biodegraded; TCE and all benzene compounds demonstrated some attenuation but degradation was unconfirmed. 1,4-dichlorobenzene demonstrated inhibition of methanogenic activity in wetland sediments.

The studies of biological activity of Robins AFB wetland sediments indicate a high potential for natural attenuation of residual-levels of contaminants that may reach the wetland system. Field investigations of the wetlands indicated that the system has many biological assets which need to be included in the overall assessment of the system. These additional assets include a robust wetland plant population, from trees and grasses, to rooted and suspended aquatic plants, and suspended and attached microbial populations, in addition to the root-zone sediments examined in these studies. In addition, acclimation by microbial populations to xenobiotic organic compounds and the induction of appropriate enzymatic systems are important considerations relative to confirmation of natural attenuation in wetland systems which were not examined in the current study.

Results of this study indicate the wetlands system has significant capacity to naturally attenuate trace to moderate levels of COCs and that this capacity should be included in any assessment of allowable discharge-levels for COCs in groundwater and surface-water discharges to the wetlands system.

Further study of natural attenuation is required in the wetlands to adequately address issues identified but unresolved in this study. These include the relative roles of aerobic and anaerobic microbial processes in the wetland sediments; rates of transport and retardation of contaminants in the subsurface clay layer; the roles of plants and suspended aquatic organisms in wetland-attenuation processes; the delineation of wetland systems into defined zones of influence (e.g. plant zone, root-zone sediments, water-column and bottom-sediments); and the mechanisms of transport and attenuation among the zones, as well as flow and contaminant-flux valves for groundwater and surface-water flows and the dilutional effects of flows from non-site areas.

1. Introduction

The scientific basis for bioremediation, the manipulation of living systems to affect desired changes in the environment, has been examined in detail and advances continue to be made and applied to solution of environmental problems. The biochemical conversion of organic pollutants to less toxic compounds or complete mineralization by bacteria and living organisms is a natural process. When considering the application of biological processes for remediation of a specific contaminated site, it is important to obtain information indicative of the potential for success prior to undertaking full-scale efforts. Each hazardous waste site, however, offers a unique set of parameters with which any potential remediation must operate. For fundamental understanding, the biochemical processes used by microorganisms to degrade organic pollutants are examined in laboratory treatability studies to provide fundamental data from controlled systems regarding potential for bioremediation. These studies are configured to evaluate the applicability of a process for a particular site and identify proper parameters for implementation, or, for more detailed testing in pilot-scale systems.

1.1. Background Site Description

The intrinsic bioremediation study was performed with sediments obtained from the Robins Air Force Base (AFB) in Warner Robins, GA. The Base is an active industrial area focused on aircraft rehabilitation, cleaning, painting, repair and is an active fuel depository for on-ground and in-flight refueling of jet aircraft. In addition to soil and groundwater from these industrial activities, numerous solid waste management units (SWMUs) are believed to have contributed to the Greater Base Industrial Area (GBIA) groundwater contamination. These SWMUs include landfills, fire-protection-training areas and storm and sewer systems. The contaminants of concern (COCs) include chlorinated aliphatic compounds, petroleum hydrocarbons, chlorobenzene and dichlorobenzenes. These contaminants were transported from sources in the GBIA directly into groundwater and are exceeding MCLs at the point of compliance (Hannah Road). These contaminants may have subsequently moved into the sediments of the wetlands. Discharge into the wetland apparently occurs from a sand/gravel aquifer where groundwater migrates upward through clay and sediment beds which overlay the sand/gravel aquifer and flow through the sediment layer into the wetland. Surface runoff also may result in the discharge of COCs into the wetland. The study focuses on presenting the behavior of contaminants flowing from the sand/gravel aquifer through the sediment layer into the wetland area.

Sediments in wetland environments can be the receptors of surface and subsurface inputs from surrounding sites and facilities and receive contaminants loadings which can affect wetland systems and any subsequent water bodies receiving wetland discharges. On the other hand, wetland sediments with bioactivity can be used to decompose certain

contaminants by natural bioattenuation. At the Robins AFB, clay/peat layers have been previously identified as being biologically active with regard to the indicated COCs (CH2MHill, 1992). Principal COCs at Robins AFB in surface water and groundwater are associated with chlorinated aliphatic compounds, petroleum hydrocarbons, chlorobenzene and dichlorobenzene. These contaminants are being transported from sources in the GBIA towards the wetlands adjacent to Robins AFB. A past summary of selected contaminants in groundwater, site soils and wetland sediments and their concentration ranges are in Table 1.2.1 (CH2MHill, 1992).

The concentrations of these contaminants in the areas of the wetlands appear to indicate some potential for dramatic reductions with respect to potential influent concentrations. Furthermore, the sediment and underlying clay-sediment layer have been identified as being biologically active with regard to the indicated contaminants (CH2MHill, 1992). TCE, a COC in GBIA at Robins AFB, was observed in an earlier study (CH2MHill, 1992) to be anaerobically degraded to cis-1,2-dichloroethylene (*cis*-DCE) in sediments with an estimated half-life of 6 to 48 days. Vinyl chloride was slowly formed as degradation proceeded but was not accumulated at high concentrations. In a TCE-contaminated groundwater flowing upward through a sediment/clay layer, significant retardation was observed due to adsorption on sediment. Since the adsorption reduced the rate of volatilization, biodegradation can become more important in the mechanisms of elimination of TCE. CH2MHill (1992) also reported that TCE is persistent, without any form of transformation, in the sand/gravel aquifer and in groundwater contacted with clay. The capability of indigenous sediment microorganisms to biodegrade other COCs was not examined (CH2MHill, 1992). The biodegradation potential and kinetics of several target compounds (e.g., acetone, phenol, benzene, chlorobenzene, 1,4-dichlorobenzene and TCE) are evaluated in this study.

1.2. Problem Statement

The overall objective of the project initiative was to investigate the approach of using wetland sediment systems for natural attenuation and intrinsic bioremediation. Sediments in wetland systems are composed of accumulated plant debris and soil in a biologically-active, aerobic/anaerobic environment. Wetland sediments can potentially be similar to the sediments of wetland-associated streams with which periodic sediment exchange occurs. The organic-rich nature of a wetland sediment system may therefore affect bioavailability and bioremediation processes. Furthermore, wetland sediments have a potential to be effective barriers to contamination of a watercourse, as well as potential contributors of natural organic matter and nutrients. Wetland systems have been designed and effectively utilized for reclamation of wastewater and have been demonstrated to be effective in stabilization of organic matter and hazardous substances (e.g., Reed *et al.*, 1988). The bioattenuation potential of the project area and the feasibility of using natural attenuation to clean up the contamination were investigated in this research.

Table 1.2.1. Contaminant Levels Associated with Robins AFB Wetland System (CH2MHill, 1992)

Contaminants	Groundwater Flow Concentration (μg/L)	Surface Water Concentration (μg/L)	Soils and Sediments Concentration (μg/kg)
TCE	10-2400	< 10	------
Total Chlorinated Aliphatic	10-9700	<10	------
BTEX (sum)	1000-34,000	5-40,000	1-70
Chlorobenzene	100-1,700	5-20	5-150
Dichlorobenzene (1,4-DCB plus 1,2-DCB)	100-1050	4-10	4-40

Major processes responsible for the reduction of contaminants or the size of a groundwater plume may include: (i) release of dissolved contaminants from the source area, (ii) transport and mixing of contaminants with groundwater, (iii) volatilization, (iv) abiotic and biotic transformations of contaminants, and (v) irreversible sorption of contaminants on sediment particles (Rifai *et al.*, 1995).

1.2.1. Fate of Organic Chemicals in the Wetland System

A simplified schematic diagram of the fate of organic chemicals in the environment is presented in Figures 1.2.1a. and 1.2.1b. (Swoboda-Colberg, 1995). However, those figures are obviously overly simplified since there are many subtle distinctions and unknowns in natural and wetland environments that are not included. For example, there are few compounds which are totally insoluble in water. Other important mechanisms determining the fate of synthetic organic chemicals are chemical and microbial transformations or degradation. In both chemical and microbial processes, compound degradation is dependent on the availability of a catalyst and on environmental conditions. Environmental factors have a dramatic effect on microbial transformations. Controlling factors are contaminant bioavailability, presence or absence of oxygen and nutrients, presence of indigenous microbial communities, temperature, pH and favorable redox conditions.

Degradation involves a number of transformations prior to mineralization. Intermediates, which may be toxic or nontoxic, can be formed during the process of biodegradation. It becomes very important to understand that for a natural environment we have to be concerned not only for the attenuation of organic chemicals, but also for the formation of intermediates during biotransformation.

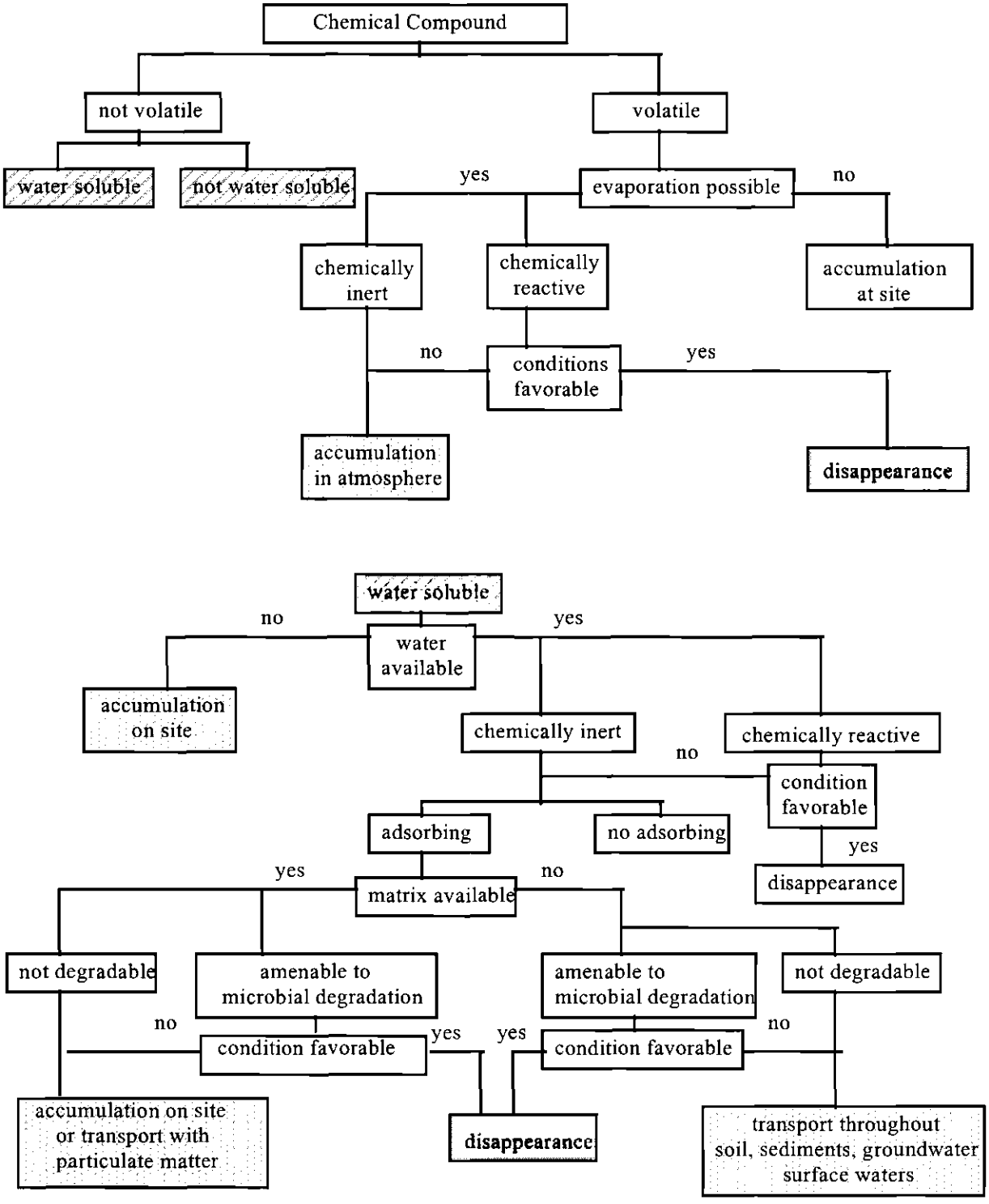


Figure 1.2.1a. Fate of Organic Chemicals in Wetland Systems (Swoboda-Colberg, 1995)

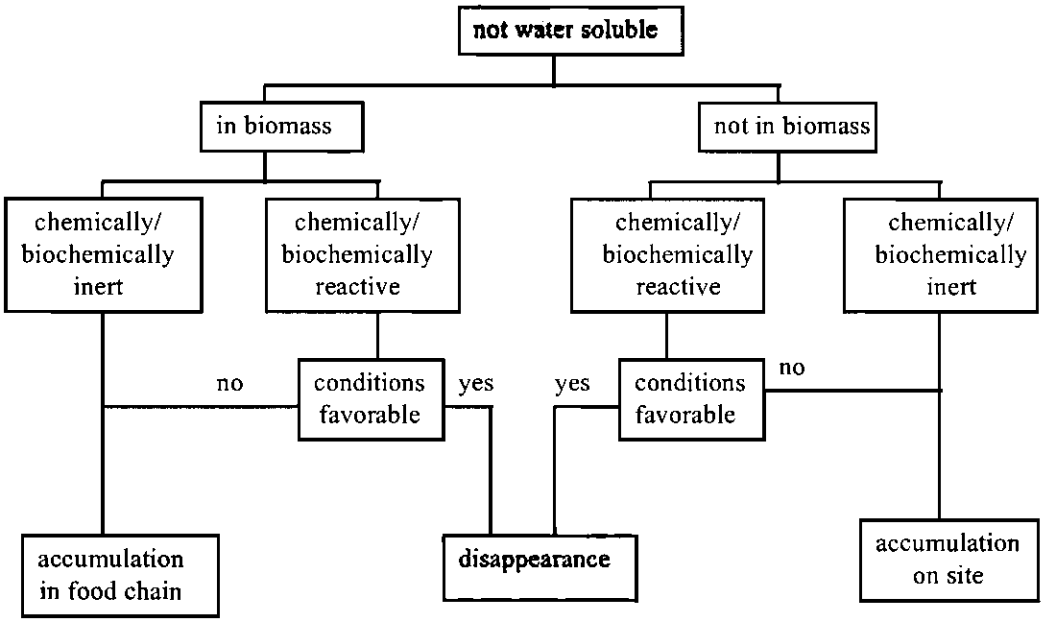


Figure 1.2.1b. Fate of Organic Chemicals in Wetland Systems (Swoboda-Colberg, 1995)

Biotransformation of organic pollutants (e.g., BTEX, TCE and chlorobenzenes) under aerobic and anaerobic environments has been investigated and advanced in the published literature. The possible biological transformation processes for the COCs using indigenous microcosms in sediments via aerobic and anaerobic pathways are briefly introduced below.

1.2.2. Aerobic Pathways and Bioprocesses

BTEX. Benzene, toluene, ethylbenzene and xylenes are referred to as BTEX compounds. Under aerobic conditions, microorganisms can readily degrade monoaromatic hydrocarbons and the pathways for metabolism of these aromatic compounds have been studied intensively. The first step in aerobic biodegradation of benzene involves hydroxylation of the ring. Bacteria employ a dioxygenase which incorporates two atoms of oxygen simultaneously into the ring (Rochkind *et al.*, 1986). Molecular oxygen is a required substrate for this enzyme. The resultant intermediate compound is a *cis*-1,2-dihydroxy-1,2-dihydroxybenzene which can then lose two hydrogens to become catechol. This pathway is generally applicable for all bacteria species studied. Under aerobic conditions, benzene, toluene, xylene and ethylbenzene can be mineralized to carbon dioxide.

The amendment of electron donors and acceptors under aerobic conditions to enhance BTEX biodegradation was studied (Bouwer, 1988; Doong *et al.*, 1995). Bouwer (1988) indicated that the addition of easily degradable carbon sources and electron

acceptors (nitrogen) may markedly enhance biotransformation of aromatic hydrocarbon compounds in a strip-pit pond. In contrast, Doong *et al.* (1995) reported that electron donors, such as acetate or glucose, prolonged the mineralization time compared to the process without electron donor amendment.

TCE. For aerobic degradation of TCE, a suitable primary substrate must exist in order to stimulate the enzyme for cometabolism and supply the carbon as well as energy sources for biodegradation. However, the bacteria that can transform TCE have a limited spectrum of required substrates spectrum, such as aromatic compounds or methane. The biotransformation of TCE may not naturally occur if soils or sediments do not contain a suitable primary substrate.

One group of microorganisms can cometabolize TCE when an aromatic compound is supplied as primary substrate and several species have been isolated and identified. One organism strain, *Pseudomonas cepacia* G4, requires toluene, *o*-cresol, *m*-cresol, or phenol for TCE degradation activity (Folsom *et al.*, 1990, Krumme *et al.*, 1993). This organism has been shown to employ a novel toluene-degradation pathway involving sequential hydroxylation of toluene at *ortho* and *meta* positions to form 3-methylcatechol (Shields *et al.*, 1989). TCE is completely degraded by this organism to CO_2, Cl^-, and unidentified, nonvolatile products (Nelson *et al.*, 1988). The bioconversion of TCE by *P. cepacia* involves the action of enzyme(s) whose natural substrates appear to be toluene and phenol. However, a degree of inhibitory interaction between the natural substrate and other chemicals serving as substrates was observed (Folsom, *et al.*, 1990). In the case of TCE degradation, in which phenol is employed as both the inducer and the substrate, competition inhibition is likely to be severe since the K_s values for both TCE and phenol are similar (5 to 10 μM). Though the exact mechanism of inhibition was not determined, a competition mechanism is suspected between the natural substrate (phenol) and TCE. At a concentration of TCE higher than 50 μM, TCE temporarily inhibited its own degradation. Similar concentration levels of inhibition were reported in pure culture (Winter, *et al.*, 1989; Wackett and Gibson, 1988) and soil microorganism consortia (Mu and Scow, 1994).

There is a wide variety of indigenous bacteria that have the ability to cometabolize TCE. The majority are aromatic-compound degraders, methanotrophic bacteria and some ammonia oxidizers. Since TCE can only be transformed by cometabolic processes, a primary substrate is required to serve as both energy and carbon supplier and enzyme inducer. The most widely used substrates of aromatic degraders are toluene and phenol. Both TCE and the primary substrates are toxic to bacteria at high concentrations. Therefore, at a contaminated site, the concentrations of TCE and aromatic compounds are equally important to assure biotransformation of TCE and high concentrations of aromatic compounds and TCE can completely shut off biotransformation. For methanotrophic bacteria, methane or formate is essential for TCE cometabolism.

Chlorobenzenes. Although a number of microorganisms can utilize halogenated benzene as a primary substrate under aerobic conditions, chlorobenzene and dichlorobenzene are considered recalcitrant for biodegradation (Nishino *et al.*, 1994). Reineke and Knackmuss (1988) described a bacterium which can utilize chlorobenzene as primary substrate and sole energy source after nine months of incubation of a benzene-degrading population with increasing amounts of chlorobenzene (CB). The original inoculum was from a mixture of many soil and sewage samples.

The general pathways of chlorobenzene degradation were studied by Reineke and Knackmuss (1988) and Nishino *et al.* (1992). Chlorobenzene is transformed to chlorocatechol followed by *ortho*-cleavage of the ring. The substituted muconic acid is dechlorinated and then the non-chlorinated intermediate products metabolized to 3-oxoadipate which enters the tricarboxylic acid cycle. This pathway is basically identical to the pathway of aerobic benzene degradation and the only difference is that chloride is eliminated from the organic compound at some point of the pathway through dehalogenation processes (Nishino *et al.*, 1992).

Dichlorobenzenes are susceptible to degradation by a number of bacteria under aerobic conditions. An *Alcaligene* sp., capable of using 1,3-dichlorobenzene as a sole carbon and energy source, was isolated from a mixture of soil and water after six months of enrichment (de Bont *et al.*, 1986). Similarly, a 1,4-dichlorobenzene degrader, another *Alcaligenes* sp., was isolated. Spain and Nishino (1987) isolated a *Pseudomonas* sp. from activated sludge which can use 1,4-dichlorobenzene (*p*-DCB) as a sole source of carbon and energy. A *Pseudomonas* sp. able to degrade 1,2-dichlorobenzene was isolated from soil and sewage after 14 months of incubation. Through selective enrichment, a *Pseudomonas* sp. using 1,2-DCB or chlorobenzene as sole source of carbon and energy was isolated (Haigler *et al.*, 1988). Metabolic pathway of 1,2-dichlorobenzene (*o*-DCB) by a *Pseudomonas* sp. was developed by Haigler *et al.* (1988). Extracts of *o*-DCB-grown cells converted *o*-DCB to 3,4-dichloro-cis-1,2-dihydroxycyclohexa-3,5-diene (*o*-DCB dihydrodiol) by a dioxygenase. *o*-DCB dihydrodiol is converted to 3,4-dichlocatechol by an NAD^+-dependent dehydrogenase. Ring cleavage of 3,4-dichlocatechol is by a catechol 1,2-oxygenase to form 2,3-dichloro-cis,cis-muconate. Chloride is then eliminated during subsequent lactonization of the 2,3-dichloro-cis, cis-muconate, followed by hydrolysis to form 5-chloromaleylacetic acid. Metabolic pathways for 1,3-dichlorobenzene (*o*-DCB) by a *Pseudomonas* sp. was similar to the pathway of 1,2-dichlorobenzene (*o*-DCB). *p*-DCB was initially converted to 3,6-dichloro-cis-1,2-dihydroxycyclohexa-3,5-diene (*p*-DCB dihydrodiol) by a dioxygenase. *p*-DCB dihydrodiol is converted to 3,6-dichlocatechol by an NAD^+-dependent dehydrogenase. Ring cleavage of 3,6-dichlocatechol is by a catechol 1,2-oxygenase to form 2,5-dichloro-cis, cis-muconate (Spain and Nishino, 1987).

Indigenous microorganisms have the advantage of being adapted to the physicochemical characteristics of a site, but they may not be able to remove the hazardous substances at reasonable rates. Chlorobenzene contaminated soil and groundwater were used to determine whether indigenous bacteria could degrade the

contaminants and compare the biodegradability with specific chlorobenzene-degrading *Pseudomonas* sp. Strain JS150 (Nishino *et al.*, 1994). The results showed that indigenous populations have a competitive advantage over inoculated pure cultures for chlorobenzene degradation.

Bouwer (1989) found that the addition of acetate, nitrogen, and phosphate markedly enhanced the rate of biotransformation of chlorobenzene in strip-pit pond. Enhanced biotransformation rates were more than an order of magnitude faster than volatilization rate and becomes the major removal mechanism. Chlorobenzene was mineralized to carbon dioxide. The presence of 0.61 g/L of sediments did not significantly affect the biotransformation and volatilization rates for aromatic compounds. Low contaminant concentrations were maintained by rapid microbial utilization to promote desorption of contaminants from the sediment. Finally, the addition of molecular oxygen is required under subsurface conditions and in soils to promote aerobic biotransformation (Bouwer, 1982; Criddle *et al.*, 1986).

1.2.3. Anaerobic Pathways and Bioprocesses

Metabolic steps in the biodegradation of hydrocarbons follow two major strategies: oxidation and reduction. Because hydrocarbons are chemically reduced and stable compounds, further reduction, while thermodynamically possible, is not a primary mode for biodegradation, even under strict anaerobic conditions. A number of reports have demonstrated that toluene, benzene, and a variety of other compounds can be biodegraded under strict anaerobic conditions by anaerobic cultures (Bossert and Compeau, 1995). The metabolic steps will be described briefly by the following.

BTEX. It was generally believed that BTEX can be degraded under aerobic or anoxic conditions but recalcitrant under anaerobic conditions (Evans and Fuchs, 1988; Doong *et al.*, 1995; Barker *et al.*, 1986). Grbic-Galic and Vogel (1987) reported that benzene and toluene can be anaerobically transformed by acclimated, mixed methanogenic cultures after a long lag phase and eventually mineralized.

The metabolic pathway for aromatic hydrocarbons under methanogenic fermentation has been established by Grbic-Galic and Vogel (1987) and Evans *et al.* (1988). ^{18}O-labeled water and ^{14}C-labeled compounds were used to delineate the possible mechanisms and enzymes involved in these unique pathways. There are three possible degradation pathways involved in toluene degradation under anaerobic environments by mixed methanogenic cultures through oxidation to cresol and benzyl alcohol. ^{18}O-labeled water showed that water was the source of oxygen for oxidation in anaerobic conditions. Further reactions included reduction of the ring of benzenoid structures, followed by ring cleavage. The flavocytochrome c. reacts with p-cresol, probably forming a quinone methide intermediate by dehydrogenation, which then adds on water to give the alcohol. The reactions involved in the anaerobic oxidation of the aromatic hydrocarbons are both endorgenic with most electron acceptors. Energy or a positive electron acceptor is therefore required. Sequence dehydrogenation, carboxylation/decarboxylation reactions,

β-oxidation of acetyl side groups, and many reactions of heterocyclic compounds are involved and require terminal acceptor for each oxidation step (Evans *et al.*, 1988).

In general, biotransformation of aromatic compounds under anaerobic conditions is not ubiquitous (Barker and Acton, 1992) and several negative results were reported. Thus, strictly anaerobic conditions may limit the possibility for degradation of toluene and o-xylene under natural conditions, especially with the co-existence of complex natural organics and co-contaminants other than BTEX.

Phenol. Phenol is usually a trace component of most petroleum products. It is sometimes difficult to determine if these compounds were present as a primary component of a hydrocarbon product or if they were intermediate products of microbial degradative processes. Under anaerobic conditions, phenol is an intermediate oxidation product of benzene metabolism. As such, the pathways of phenol degradation may be closely associated with other pathways for aromatic hydrocarbon degradation (Chapelle, 1992).

TCE. TCE transformation under anaerobic conditions was studied as an intermediate of PCE dechlorination. Under anaerobic environments, PCE is transformed to ethane via TCE, *cis*-1,2-dichloroethylene (*cis*-1,2-DCE), chloroethylene (VC), and ethylene (Holliger *et al.*, 1993; Freedman, 1990). However, most biotransformations resulted in accumulation of less-chlorinated ethylenes, especially VC. A highly purified enrichment culture (PER-K23) which couples the reductive dechlorination of PCE to growth was reported by Holliger *et al.* (1993). This culture did not grow in the absence of PCE or TCE. PCE or TCE served as an electron acceptor with H_2 as an electron donor. Experiments with $^{14}CO_2$ revealed that CO_2 was incorporated only via heterotrophic CO_2 fixation under the growth conditions tested. The rest of the carbon was apparently assimilated from organic compounds present in the fermented yeast extract added. Maymó-Gatell *et al.* (1995) enriched an anaerobic culture which, by using methanol as an electron donor and H_2 as an electron acceptor, dechlorinated PCE to VC and ethane. Carter and Jewell (1993) indicated that, in general, anaerobic treatment of PCE and TCE is an incomplete process if the end-product is VC. Further treatment to remove DCE and VC would be required to meet effluent limits for these compounds. A primary substrate is required to serve as both energy and carbon supplier and enzyme inducer. Glucose, yeast extract and propionate have been used as primary substrates for biotransformation of cDCE to ethylene under anaerobic conditions. Results indicated that cDCE was almost completely dechlorinated to ethylene in the presence of glucose, yeast extract or propionate. The transformation rate was somewhat lower in the culture fed with hydrogen (Komatsu *et al.*, 1994).

Chlorobenzene and 1,4-dichlorobenzene. The behavior of chlorobenzenes in anaerobic aquifers is complex (Chapelle, 1992). While reductive dehalogenation has been closely studied using halobenzoates as model compounds, much less is known about the behavior of chlorobenzenes. Based on the benzoate model, however, reductive

dehalogenation would be expected to be the predominant degradative process. However, this behavior has not been observed in field studies. In one study of a sewage effluent-contaminated anaerobic aquifer, DCB was shown to persist for a minimum of 20 years (Barber, 1988). However, because denitrification was the predominant terminal electron-accepting process, it is difficult to generalize these results for other terminal electron-accepting processes, such as Fe (III) reduction or methanogenesis.

1.2.4. Toxicity Levels for Site Contaminants

Organic constituents in groundwater plumes may be toxic to microorganisms and therefore inhibit biodegradation processes. Tang *et al.* (1992), Blum and Speece (1991), Hermens *et al.* (1985) have presented data on the sensitivities of various microorganisms to toxicants. TCE was, for example, found to transiently inhibit its own degradation using pure culture at concentration of TCE higher than 50μM (6.6 mg/L) (Folsom *et al.*, 1990; Winter *et al.*, 1989; Wackett and Gibson, 1988). A list of toxic levels (inhibitory concentrations [IC_{50}]) for COCs is presented in Table 1.2.2.

1.3. Objectives

The project is focused on the ultimate development of an engineering approach to the use of natural attenuation processes in bioremediation of contaminated sites at Robins AFB using wetland sediments. Fundamental knowledge of a complex system with soil, sediment and groundwater systems and multiple pollutants is a critical issue of this research. The objectives of this initial project to be focused on natural bioremediations in wetland were to:

1. Characterize physical, chemical and biological properties of wetland sediments and understand their potential role in natural attenuation.

2. Examine general microbial activities in wetland sediments under aerobic, and anaerobic environments to establish the potential for biotransformation.

3. Determine biodegradation rates and for target contaminants under aerobic and anaerobic conditions with controlled laboratory systems and wetland sediments.

4. Determine biodegradation kinetics for wetland sediments under aerobic and anaerobic conditions for selected contaminants representative of the site.

5. Develop a rationale for the use of natural attenuation remediation and present a next-level approach to confirmation of the efficacy of natural attenuation processes using wetland sediments.

Table 1.2.2. Literature citations for inhibition and toxicity levels of targeted organic contaminants.

Target Compounds	Solubility (mg/L)	Microorganisms	IC50 (mg/L)	Remarks	Reference
Benzene	1789	Nitrobacter	957	QSAR Analysis	Tang et al. (1992)
		Nitrosomonas	13	Ammonia use, 450 mg/L VSS*	Blum and Speece (1991)
		Methanogens	1200	Gas Production, 900 mg/L VSS	Blum and Speece (1991)
		Aerobic Heterotrophs	520	Oxygen consumption, 200 to 1800 mg/L VSS	Blum and Speece (1991)
		Microtox	75	Bioluminescense	Blum and Speece (1991)
		15 minute Microtox	1500		Hermens et al. (1985)
		Microtox	50		Vasseur et al. (1986)
		Microtox (20 C)	>150		Vasseur et al. (1986)
Chlorobenzene	503	Nitrosomonas	0.71	Ammonia use, 450 mg/L VSS	Blum and Speece (1991)
		Methanogens	270	Gas Production, 900 mg/L VSS	Blum and Speece (1991)
		Aerobic Heterotrophs	310	Oxygen consumption, 200 to 1800 mg/L VSS	Blum and Speece (1991)
		Microtox	9.4	Bioluminescense	Blum and Speece (1991)
		15 minute Microtox	15		Hermens et al (1985)
		0.5 hour Resazurin reduction	410-470		Thompson et al. (1986)
1,4-Dichlorobenzene	59.88	Nitrobacter	[227]	QSAR Analysis	Tang et al. (1992)
		Nitrosomonas	[86]	Ammonia use, 450 mg/L VSS	Blum and Speece (1991)
		Methanogens	[86]	Gas Production, 900 mg/L VSS	Blum and Speece (1991)
		Aerobic Heterotrophs	[720]	Oxygen consumption, 200 to 1800 mg/L VSS	Blum and Speece (1991)
		Microtox	4.3	Bioluminescense	Blum and Speece (1991)
		0.5 hour Resazurin reduction	[180-220]		Thompson et al. (1986)
Trichloroethylene	1198.4	Nitrobacter	921	QSAR Analysis	Tang et al. (1992)
		Nitrosomonas	0.81	Ammonia use, 450 mg/L VSS	Blum and Speece (1991)
		Methanogens	13	Gas Production, 900 mg/L VSS	Blum and Speece (1991)
		Aerobic Heterotrophs	130	Oxygen consumption, 200 to 1800 mg/L VSS	Blum and Speece (1991)
		Microtox	960	Bioluminescense	Blum and Speece (1991)

Table 1.2.2. (cont'd) Literature citations for inhibition and toxicity levels of targeted organic contaminants.

Target Compounds	Solubility (mg/L)	Microorganisms	IC50 (mg/L)	Remarks	Reference
Acetone	7.84E+05	Nitrosomonas	1200	Ammonia use, 450 mg/L VSS	Blum and Speece (1991)
		Methanogens	50000	Gas Production, 900 mg/L VSS	Blum and Speece (1991)
		Aerobic Heterotrophs	16000	Oxygen consumption, 200 to 1800 mg/L VSS	Blum and Speece (1991)
		Microtox	16000		Tarkpea et al. (1986)
		6 hour Growth P. putida	594		Slabbert (1986)
		15 minute Microtox	21000		Hermens et al (1985)
		Biodegradation Inhibition	35545		Vaishnav (1986)

* VSS = volatile suspended solids
[ ]: IC50 higher than solubility

2. Sampling Summary

Investigation of natural-attenuation-based remediation with wetland sediments at Robins AFB required collection of representative sediment samples for investigation with laboratory systems. Initial site visits were focused on establishing a clear delineation of the sediment layer in the wetlands and the clarification of the status of the "peat" soil layer at the site. The sampling events and sediment-handling procedures employed at the site are presented below.

2.1. Sampling Locations

Samples were taken from various points within Robins AFB wetlands east of the industrial area. Four sampling trips (21 November 1995; 07 March 1996; 07-08 April 1996; and 03 September 1996) were taken to collect wetland sediments, sands, clays and groundwaters.

On 21 November 1995, samples were taken from three locations for preliminary analyses and development of sampling and laboratory techniques. Preliminary experiments performed with November 1995 samples to develop techniques are not included in this report but the approach to analysis was developed with these samples. The November 1995 sites are outlined below to indicate, in part, areas included in the assessment of the reported peat layer and to concurrently develop field sampling events with Rust staff, who attended all sampling trips.

Prelim SITE A: Site is located in a drainage ditch west of Light Service Road. Depths of 0-1ft. consisted of dark organic matter, with depths greater than 1ft. consisting of wetland soil mottled with a clay texture.

Prelim SITE B: Site is located in a marsh-like environment consisting of a layer of cane grass atop standing water. The site is located southwest of the Second Street and Light Service Road intersection. Site B is typified as a ditch near the creek bed leading to Site A (above). Soil at depths of approximately 1ft. is defined as clay.

Prelim SITE C: Site is located south of Horse Creek Road. At depths of less than 1ft, soils consisted of clay.

A reconnaissance visit of March 1996 was conducted to establish sampling locations. Wetland samples were taken in April 1996 at redefined wetland locations. These sediments were used in laboratory bioactivity assessments of the wetland system. Sediment samples are outlined in the sediment sample location map (Figure 2.1.1) and are listed therein as NA-SED-01 through NA-SED-09. The sampling sites are defined as:

SITE-01: East of well cluster LF2-15/16/17, approximately 30 meters east of Hannah Road

SITE-02: South of well R15-3WP; 30 meters south into the wetlands from the SAC parking-area and wetland border.
SITE-03: East of well cluster LF2-18/19/20 at various locations (as defined later).
SITE-04: South of well R15-4WP, approximately 10 meters south of Horse Creek Road in a former wastewater discharge channel.
SITE-05: South of well R15-4WP at 30 meters south of Horse Creek Road.
SITE-06: East of well cluster LF4-18/38/39, approximately 30 meters east of Hannah Road.
SITE-07: East of well cluster LF2-1/10/11, at 30 meters west of Light Service Road.
SITE-08: East of well cluster LF2-1/10/11, near 30 meters east of Light Service Road at an island-type mound with relatively abundant vegetation (compared to the surrounding area of SITE 07 which contained a heavy straw bed at the surface).
SITE-09: North of the northwestern most boundary of Robins AFB in a background area outside of any known contaminant plume.
GW: Groundwater samples were collected from the LF2-18 groundwater monitoring well and thus denoted as NA-RAFB-0496-GW-LF218.

In an effort to focus on one area of potential contamination, samples were collected in September 1996 within SITE-03, as defined in April sampling. The September 1996 samples are included in the sediment sampling location map (Figure 2.1.1) and are differentiated from April samples by a subset number extension added to SITE-03. These subsets are taken from SITE-03 which has been divided into three sampling subsites covering 100 square meters. These subsites are described as:

SITE-03-01: East of LF2-18, 50 meters east into wetlands.
SITE-03-02: East of LF2-18; 10 meters south of SITE-03-01
SITE-03-03: East of LF2-18; 10 meters west of SITE-03-01

2.2. Sampling Technique

The main purpose of sampling was to collect wetland sediments, sands and clays, and groundwater samples from various areas and depths for laboratory physicochemical and microbial studies. Sediment samples (i.e., SED) were generally collected from immediately beneath an overlying root zone. The zone was penetrated with a hand shovel to reveal an underlying sediment with high moisture content. The sediment was too fluid for hand-auger retrieval, therefore shoulder-glove hand retrieval was required. Over approximately a one-meter depth, samples were collected manually and immediately transferred to autoclaved sample containers and sealed.

Clay and sand samples were collected at depths greater than one meter. For clay and sand samples, a PVC casing was driven below the fluid-like sediments to a dense

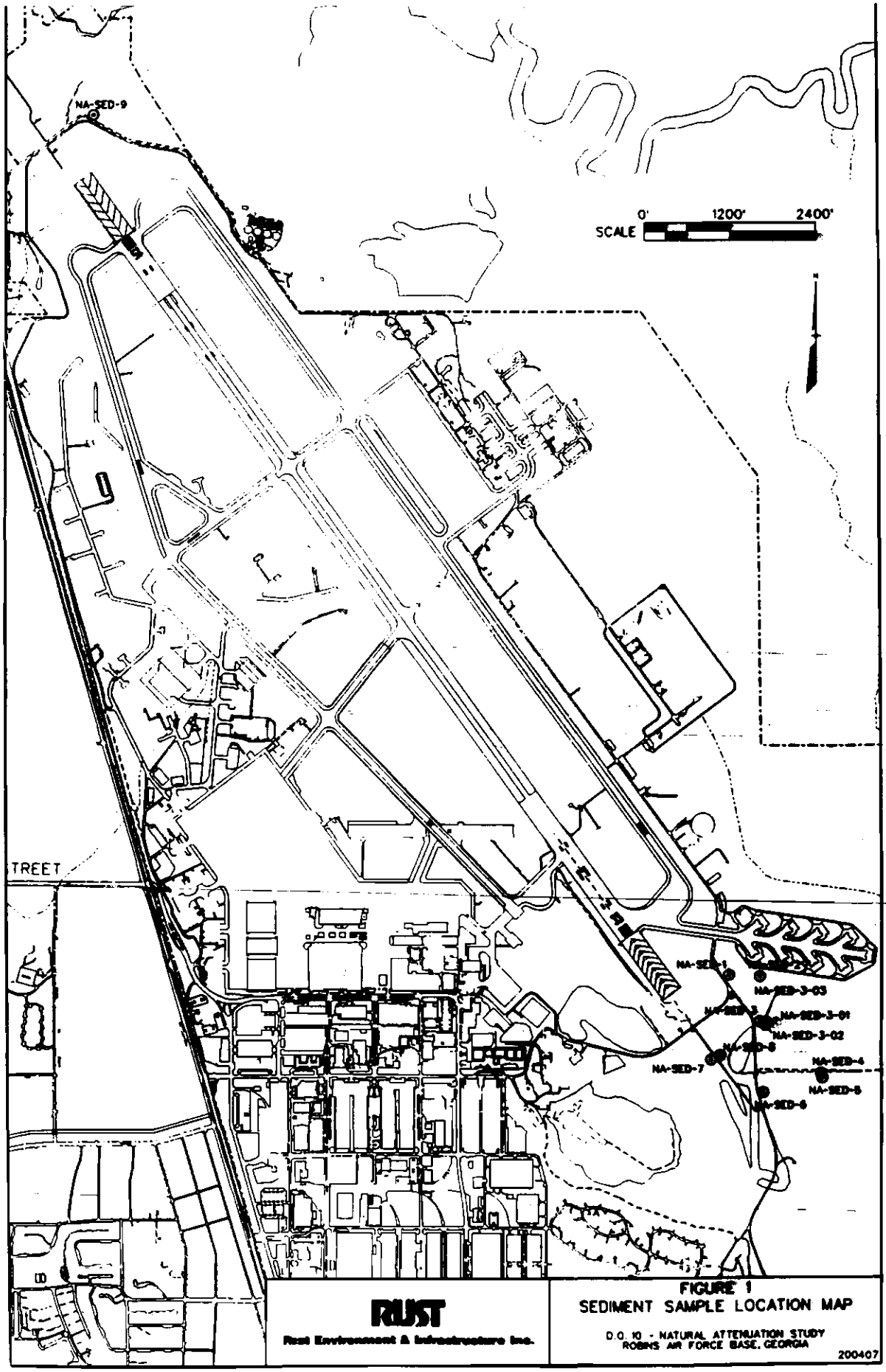


Figure 2.1.1. Sediment sample location map

layer of sand and clay. Clay and sand samples in the casing were retrieved with a hand auger and transferred to sample containers.

Groundwater samples were taken at the LF2-18 well. Samples were taken with a groundwater hand bailer by RUST personnel.

At least two samples are taken for each sediment, soil, and groundwater sampling site, and sediment samples frequently included 20 to 30 quart-volume containers. After sample collection, samples were systematically numbered and labeled with field measurements. The numbering system represents the sampling date, the sampling location and the sample number. For instance, sediment samples taken from SITE-01 in April 1996 were marked as NA-RAFB-0496-SED-01. Field measurements such as pH, temperature, ORP, and DO were also included on the label. The SED indicator for this sample reflected a sediment sample from the upper 1-m depth. Samples of clay and sand were labeled in a similar manner as above using "CLAY-01" and "SAND-01" in the labeling scheme.

Safety apparel such as chest waders, long rubber gloves, and latex gloves were utilized to prevent personal contact with potentially contaminated samples. Decontamination of sampling apparel, sampling equipment, and analytical equipment was exercised after sample collection at each site to prevent cross-contamination between sites. This was done using procedures established by RUST personnel at the Base decontamination facility after each sampling.

2.3. Field Measurements

Since the natural status of wetland sediments directly impacts environmental and physiological constraints that affect biodegradation, several on-site measurements of wetland sediments were taken to be reflective of the conditions associated with the natural environment of the sediment materials. The measurements included pH, dissolved oxygen (DO) level, and oxidation-reduction potential (ORP). The descriptions of each measurement techniques are presented in Section 3.1.

2.4. Sample Preservation

Collected samples were transferred to autoclaved Mason® canning jars (1 pint and 1 quart volumes), premarked with identification numbers. After sampling, the outside of the sample container was cleaned and labeled with site measurements and information. The jars were double bagged in Ziploc® storage bags and temporarily stored in an ice-packed cooler for transport to Daniel Laboratory, Georgia Institute of Technology. At Daniel Laboratory, the samples were processed as described below and stored at 4°C until subsampled for investigation.

2.5. Pretreatment of Sediment Samples

2.5.1. Sediment Homogeneity

Sediment homogenization involved screening and combining sediments sampled from the same site within 5 days of collection. Inside a nitrogen-filled glove bag, samples were removed from sample containers and sieved through a 0.25-cm screen. Large particles such as twigs, stones, and leaves were removed from the sediments. All sediments from a particular site were combined, manually blended and retransferred to Mason® jars prepurged with nitrogen and maintained at 4°C to minimize bioactivity. Combining the sediments resulted in a more homogeneous sample to produce more consistent experimental results at different subsampling times.

2.5.2. Pore Water Extraction

Sediment pore water is defined as the water filling the space within sediment particles and not held by surface forces to sediment particles. Usually, squeezing and centrifugation techniques are used to collect pore water. Centrifugation was used in this study to obtain pore water from sediments. Three hundred grams of wet sediment were taken from Mason jars and transferred into 500 mL polypropylene centrifuge tubes and the centrifuges tubes were closed with tight-fitting snap caps with O-rings of silicon rubber. Centrifugation was performed at 10,000 rpm for 20 minutes at 4^0C and about 100 mL of pore water was collected. Part of each supernatant was poured out and analyzed for pH and alkalinity. The rest of supernatant was filtered through rinsed 0.45-μm membrane filters and immediately acidified to pH<1 in glove bag, except the subsamples for TDS analysis. The samples were then stored in 250 mL polypropylene bottles at 4^0C in preparation for COD, TOC, ammonia and anion analyses.

3. Experimental Methods

Experimental methods were developed for understanding the behavior of active wetland sediments under aerobic and anaerobic environments. The first phase of experiments focused on analyzing physicochemical and biological characteristics of wetland sediments.

The second phase of experiments was to evaluate general microbial activity of wetland sediments using easily-degraded organic compounds as electron donors for microbial metabolisms. This phase of study was to determine the potential of wetland sediments for biodegradation of organic compounds.

The third phase of experiments was conducted to assess the capabilities of indigenous microorganisms in wetland sediments to biodegrade specific site contaminants including acetone, phenol, benzene, chlorobenzene, 1,4-dichlorobenzene and trichloroethylene under aerobic and anaerobic environments.

The fourth phase of study was to determine the biodegradation kinetics of wetland sediments using the kinetic approach as stated below.

3.1. Analytical Methods

All analytical methods used in this study are summarized in this section, including the analytical methods for measurements of physicochemical characteristics of wetland sediment samples and water and pore-water samples in the field and the laboratory. In the field, pH, dissolved oxygen (DO) levels, oxidation-reduction potential (ORP) and temperature of wetland sediments were measured at various depths and sites. In the laboratory, pH, total organic carbon (TOC), chemical oxygen demand (COD), alkalinity, ammonia, a series of anions and total dissolved solids (TDS) were measured in groundwaters and in pore-water samples which were obtained after pretreatment of sediment samples. pH, water content, organic content and total carbon (TC) were measured for sediment solids. In addition, instrumental methods and sampling preparation procedures for GC analyses and scintillation counting are included below. These measurements were used for establishing the fate of specific site contaminants in wetland sediments.

pH. A pH/mV/^{0}C meter (HI9025C, Hanna Instruments) was calibrated against 4.01 and 7.00 buffer solutions and used for field measurements. The laboratory pH probe (Orion 420A) was calibrated against the 4.01 and 7.00 pH buffer solutions. Pore water and groundwater subsamples were prepared (section 2.5.2) and transferred into 17 mL scintillation vials for pH measurements. Sediment solid samples were prepared by diluting 4.0g (+/- 0.01g) of sediment solids with 4.0 mL (+/- 0.01) of deionized water. The sediment/deionized water mixture was stirred and allowed to acclimate for 30

minutes. After acclimation, pH was measured. Samples were prepared and measured in duplicate.

Temperature. Temperature measurements in the field were taken with a temperature probe which was connected to a pH/mV/^{0}C meter (HI9025C, Hanna Instruments). The temperature probe was inserted into wetland sediments by hand at various depths and sites to get representative data.

Dissolved Oxygen (DO). Dissolved oxygen (DO) levels in pore water of each sampling site were taken with a dissolved oxygen meter. The DO probe connected to the DO meter was calibrated according to site elevation and the temperature of a deionized water sample saturated with oxygen. The temperature and site elevation were then converted to an appropriate DO reading according to DO meter conversion charts. The DO meter was then used to measure DO at various sites and depths.

Oxidation-Reduction Potential (ORP). The pH/mV/^{0}C meter (HI9025C, Hanna Instruments) was used to take ORP measurements in the field at various sites and depths.

Water Content. Three replicates of 10-g subsamples were transferred to dry, preweighed aluminum pans and weighed. Samples were then dehydrated overnight in a 105°C oven. After dehydration, samples were transferred to a desiccator and allowed to cool for at least 1 hr. Samples were then weighed again to determine total water loss during dehydration. The percent moisture was calculated as the difference of the final and initial weights divided by the initial weight.

Organic Content. After drying at 103°C, samples and aluminum pans (from water content analysis) were placed into a 550°C furnace for 2 hours. Aluminum pans and samples were stored in desiccators until samples reached a temperature equilibrium with ambient air. The weight of pans and samples were taken and recorded. The weights before and after burning were used to determine the percent of volatile organic matter in each sample. Samples were conducted in triplicate.

Total Organic Carbon and Total Carbon. Analytical blanks and pore-water subsamples were transferred in 5 mL-volumes into TOC tubes for analysis with Shimadzu SSM TOC system. Total carbon analysis was performed on each sediment in triplicate. With a mortar and pestle, a sediment sample was finely ground with a portion used for moisture analysis and another portion used for TOC analysis. For TC analysis, samples were injected into an 800°C furnace. The instrument TC data were normalized to the solids content of the sample to establish units of gC/g dry sediment.

Chemical Oxygen Demand (COD). Procedures for COD measurements followed Standard Methods (APHA, 1995). Pore water subsamples (2 mL) were added to standard COD ampules (20-900 mg/L) and incubated at 150^{0}C for 2 hours. Adequate time was allowed to cool in the dark before titration with 0.06 M ferrous ammonium sulfate (FAS).

Three replicates and one blank were performed for each pore-water subsample. Sediment samples were analyzed in a similar manner using wet-mass (e.g., milligram levels) samples of sediments in COD (1.5g/L) ampules.

Alkalinity. Alkalinity of pore water subsamples was determined by titrating a water subsample (15 mL) with standardized sulfuric acid (1mM) to a pH 4.5 endpoint. Titration was conducted at room temperature with a calibrated pH meter.

Ammonia. The purpose of measuring ammonia (NH_4) was to quantify the bioavailable nitrogen in sediment slurry in order to evaluate the adequacy of inorganic nutrients during bioremediation. An ammonia-selective electrode method using a known-addition technique was used. The ammonia-selective electrode (Orion Research, Inc.) used a hydrophobic gas-permeable membrane and dissolved ammonia ($NH_{3(aq)}$ and NH_4^+) in pore water subsample was converted to $NH_{3(aq)}$ by raising pH to above 11 with a strong base. $NH_{3(aq)}$ then diffused through the membrane and changed the internal solution pH which was sensed by a pH electrode. Potentiometric measurements were made with a pH meter having an expanded millivolt scale. In this study, 0.1 M NH_4Cl solution was prepared as stock solution and was diluted to 2mM NH_4Cl solution as a standard. Ammonia concentrations of pore water samples were determined using a common standard-addition technique.

Anions (nitrate, sulfate, phosphate, and chloride). The concentration of phosphate, nitrate, sulfate and chloride were analyzed using Dionex DX-100 ion chromatography. Standard solutions containing a mixture of phosphate, nitrate, sulfate and chloride were prepared at various concentrations. The signals of peaks were plotted with concentrations and determined the calibration curve of each anion. The standard solutions were stored at 4°C and taken out for injections before and after sample analyses. The program parameters for determining concentrations of anions were as following: flow rate, 2.0 mL/min; pressure, 1650 psi; analog range, 100 μs; injection volume, 1mL.

Total Dissolved Solid. Total dissolved solids (TDS) were determined by drying 10 mL, pore-water subsamples in triplicate at 105°C.

Scintillation Counting. [^{14}C]-labeled organics were measured using samples collected from an anaerobic bioreactor. All [^{14}C] samples were mixed with xylene-based scintillation liquid (1:5 (v/v)) and were counted by Beckman LS6500 scintillation counter. Quenched standards run weekly to produce a standard curve were used to correct for quenching. DI water in scintillation liquid (blank) was measured to determine the background activity.

Gas Chromatography. Production of CH_4 from anaerobic bioreactors was determined by withdrawing headspace samples (0.1 mL) and then injected them into a gas chromatograph (GC-TCD; HP 5890 Series II). Liquid samples which were extracted into organic solvents were analyzed by GC analyses (GC-FID and GC-ECD, HP 5890 Series II Plus). The analytical methods, instrumental conditions for analyzing each

specific site contaminant and retention time of each specific site contaminants are listed in Tables 3.1.1. and 3.1.2.

GC-TCD		
Inlet Temperature	50	
Detector Temperature	90	
Oven Temperature	30°C for 5 min	
Pressure	25 psi	
Column Type	Packed Column	
Column Length	10.0 m	
Column Diameter	0.53 mm	
GC-FID		
Inlet Temperature	160	
Detector Temperature	220	
Oven Temperature	37°C for 5 min	10°C/min to 120°C for 3 min
Pressure	10.0 psi	
Column Type	Capillary DB-5	
Column Length	30.0 m	
Column Diameter	0.32 mm	
GC-ECD		
Inlet Temperature	220	
Detector Temperature	250	
Oven Temperature	40°C for 3 min	5°C/min to 200°C for 5 min
Pressure	19.6 psi	
Column Type	Capillary DB-5	
Column Length	30.0 m	
Column Diameter	0.32 mm	

Table 3.1.1. Analytical method of specific site contaminants using GC-FID/ECD

	GC-detector	Retention Time (min)
CH_4	GC-TCD	3.98
Benzene	GC-FID	2.82
Chlorobenzene	GC-FID	7.09
Phenol	GC-FID	10.40
1,4-Dichlorobenzene	GC-ECD	8.95
TCE	GC-ECD	1.84

Table 3.1.2. Retention time of specific site contaminants on GC-FID/ECD

3.2. MPN Study

The enumeration of microorganisms serves as an indicator for the biodegradation potential of a contaminated soil. Most-probable-number (MPN) is a method to enumerate bacteria in water or sediment samples by growing them after suitable dilution. Typically, the MPN value is determined from the number of positive tests, noted by turbid growth or gas production, in a set of five replicates made at nine dilutions. After incubation, the number of tubes of each dilution showing a positive microbial response is recorded. The bacterial density is predicted as the value most likely to give the observed distribution of positive and negative values. Estimation of the MPN is based on the Poisson distribution for extreme value (Tchobanoglous and Schroeder, 1987). *Most Probable Number Calculator,* v.4.02 (Klee, 1996) was used to calculate the MPN of sediment samples.

In this study, sterilized yeast broth composed of yeast extract, soyton, glucose and natural-soil-mineral extract was used as a growth medium. Natural-soil-mineral-extract was pore water extracted from mixture of wetland sediments plus DI water at 1 to 1.5 (kg/L) ratio. Ten milliliter of sterilized stock solution of yeast extract broth (Table 3.2.1) was added into 990 mL of natural soil mineral extract to make a sterilized yeast broth.

Component	Concentration (g/100 mL)
Yeast Extract Stock	0.025
Soyton Stock	0.025
Glucose Stock	0.025

Table 3.2.1. Stock solution of yeast extract broth

Ten grams of wetland sediment was transferred into a 150-mL media bottle filled with 90 mL of sterilized broth. The sediment slurry was well mixed using a vortex mixer for ten minutes and 10 mL was transferred into each of five test tubes to make the first dilution of 10g/100mL (0.1g/mL). The next step was to transfer a 1-mL sample from the first dilution to each of five test tubes containing 9-mL yeast soil extract to make a second dilution of 0.01g/mL. This step will be repeated until the ninth dilution of 10^{-9} g/mL was made. The inoculated tubes were incubated at ambient temperature. The turbidity due to the growth of cells was a positive reaction. The results for each dilution were reported as a fraction, with the number of positive tubes over the total number of tubes. The positive results were recorded every 24 hours until the positive results remain unchanged for another 24 hours. Estimation of bacterial numbers using the final test results can be obtained by inputting these results into the MPN calculator program. The method for enumeration of the bacteria was identical for aerobic and anaerobic studies. For maintaining the reduced environment of anaerobic study, deoxygenase was added into lactose soil extract before inoculation.

3.3. Microbial Activities Using Easily-Degraded Organic Compounds

Bacterial densities in wetland sediments were estimated with MPN. The following step, given the presence of high bacterial counts, was to evaluate the potential of indigenous bacteria in sediments to biodegrade xenobiotic organic substrates. In this study, easily degraded organic compounds were used to screen general microbial activities under aerobic and anaerobic environments. Oxygen exertion in aerobic respirometers and CH_4 production in anaerobic serum bottles were used as indicators of microbial activity under aerobic and anaerobic environments, respectively. The microbial activity of wetland sediment without addition of any xenobiotic organic compounds were tested as sediment controls.

3.3.1. Aerobic Studies

Field data and observations suggest that aerobic microbes in Base sediments can contribute to biodegradation of contaminants. Field dissolved oxygen levels were below saturation, but near the sample surface, dissolved oxygen was present. Also, a fibrous root zone was evident at all sites and samples contained root portions which could serve as oxygen aerators. Laboratory MPN experiments also showed the presence of aerobic microorganisms at significant levels. Thus, evidence suggested the presence of an aerobic environment and aerobic microorganisms which can influence biodegradation in the natural environment.

Respirometric analysis is useful in assessing the general bioactivity of a microbial population and the biodegradation of specific contaminants. An aerobic respirometer is used to directly measure the oxygen consumed by microorganisms from an oxygen-enriched environment in a closed vessel under conditions of constant temperature and agitation. The respirometer measures microbial respiration rates which can be used stoichiometrically to assess substrate usage for microbial respiration.

System Configuration. The N-CON Respirometer was used to measure oxygen utilization by microorganisms in a closed reactor system. The respirometer reactor contained sediment, microorganisms on sediment particles and those suspended in pore water, mineral sources, and, where applicable, a series of substrates. The reactor was sealed with an o-ring seal and reactor cap to ensure a closed system. This closed system utilized oxygen and substrate to produce carbon dioxide, water and substrate by-products. Carbon dioxide produced during microbial degradation was removed from the headspace gases by a potassium hydroxide trap contained within the closed reactor. The reactor cap was also attached to a connection tube which joined the reactor with the respirometer detection devices (Figure 3.3.1).

Inside the respirometer system, the reactor was placed on a magnetic stirring apparatus in a water bath at 25°C. The closed system was connected to the pressure detection device which controls the oxygen metering device. The oxygen metering device was supplied by a regulated tank of pure oxygen. A decrease in system pressure triggers oxygen injection into the reactor to maintain constant pressure. This oxygen injection is output to an input/output interface from which it is be displayed on a computer screen or output to a printer (Fig. 3.3.2).

Experimental Procedures. For each sediment, oxygen exertion was measured for a control slurry which contained sediment and media solution (Table 3.3.1) and a substrate slurry. Slurries for the respirometers were prepared at approximately 50 g/L on a dry sediment weight basis using a media solution (Table 3.3.1). These slurries were allowed to acclimate at 25°C for approximately 1 hour. After incubation, each reactor temperature was measured to insure consistent temperature at respirometer starting time. Each reactor was then spiked with 20 mg/L (on an O_2 basis) of each of the five substrates to a total of 100 mg/L (on an O_2 basis) addition to the sediment slurry. The reactors were then connected to a respirometer for a measurement of oxygen exertion.

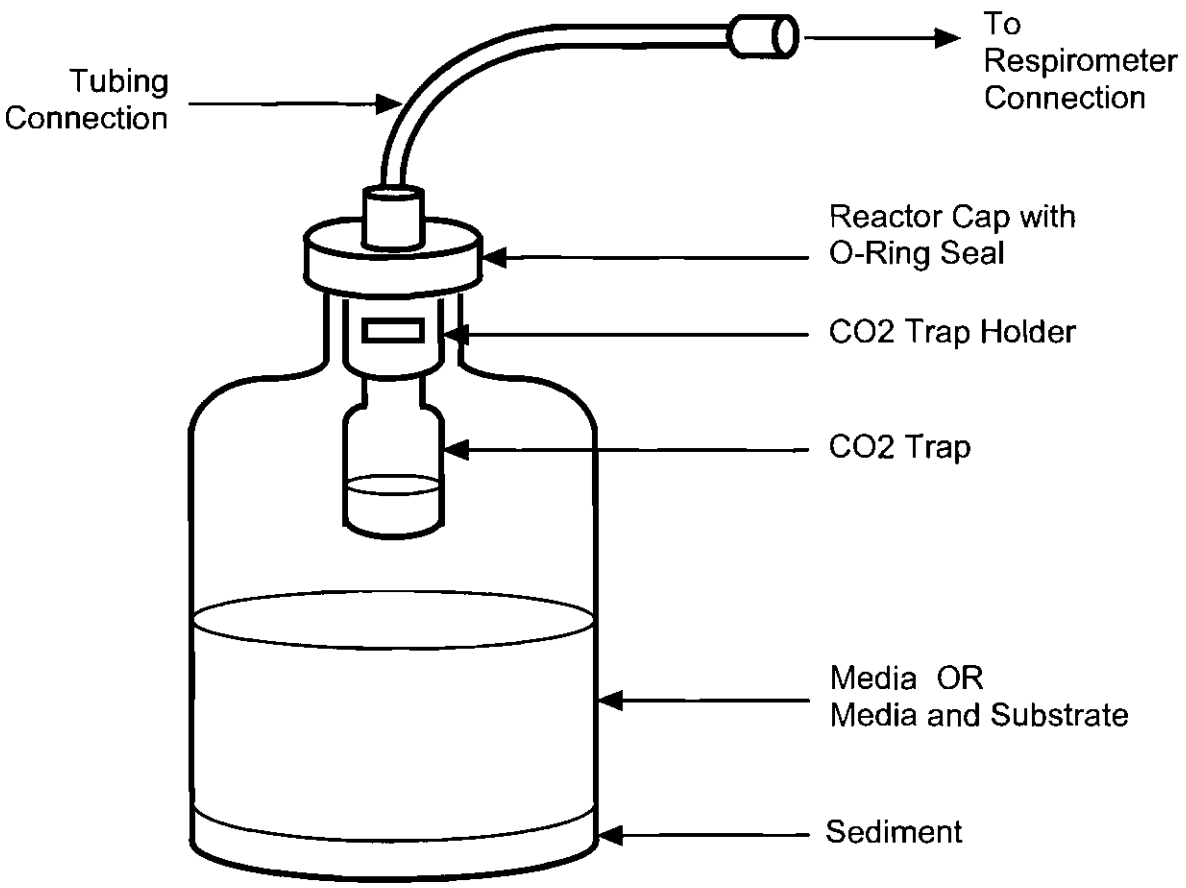


Figure 3.3.1. Respirometer Reactor for Aerobic Biodegradation Studies

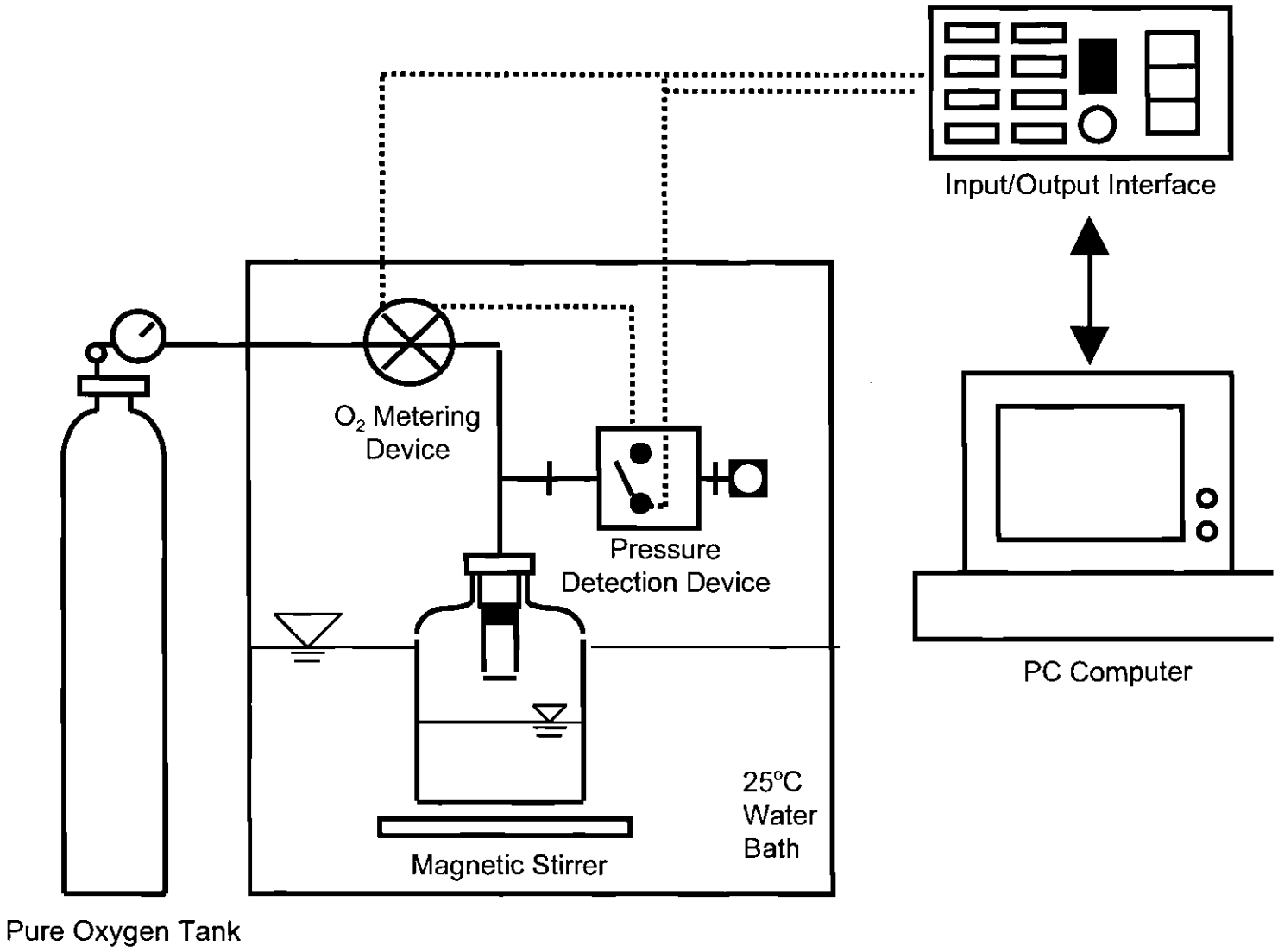


Figure 3.3.2. Configuration of N-CON Respirometer System

Chemical	Concentration (mg/L)	Chemical	Concentration (mg/L)
Mineral Sources		**Phosphate Buffer**	
$NH_4(SO_4)$	1310	KH_2PO_4	270
$CaCl_2.2H_2O$	85.5	K_2HPO_4	350
$MgSO_4$	59.2	**Trace Metals**	
$FeSO_4.7H_2O$	28	$ZnSO_4.7H_2O$	0.1054
$MnSO_4.H_2O$	0.427	$CuSO_4.5H_2O$	0.0557
H_3BO_3	0.05	$NaMo_4.2H_2O$	0.01
		$CoCl_2.6H_2O$	0.5
		$NiCl_2.6H_2O$	0.05
		$NaSeO_3$	0.05

Table 3.3.1. Media solution for Aerobic Microbial Degradation

Initial slurry percentages were based on previous data for a wet sediment-sample set. Since moisture content with each subsample changed, moisture content was taken with each subsample and used to calculate exact slurry percentages after initiation of reactor systems on a wet-mass basis. Hence slurry concentrations varied slightly around the target value of 50g/L (i.e., 5%) but were known for each reactor.

Oxygen Exertion Measurements. Respirometry measures oxygen uptake continuously over time. Oxygen exertion measurements at programmed increments of time are taken in correlation with the amount of oxygen uptake by microorganisms in the system. The initial pressure of the closed system is measured and the respirometer is programmed to detect pressure changes every 12 minutes. When internal pressure decreased, an oxygen volume is added into the system to maintain initial pressure. From the volume of oxygen added, the oxygen exerted, as a mg/L-hr rate, is calculated. The injection volume and rates were recorded in spreadsheet format for additional analysis. A plot of net oxygen exertion (mgO_2/L) vs. time (hours) was generated from these data. These plots showed the general bioactivity of the aerobic microorganisms in the system and were the basis for kinetic analysis.

3.3.2. Anaerobic Studies

Shelton and Tiedje (1984) suggested a general test to quantify anaerobic biodegradation potential using *p*-cresol, phthalic acid, ethanol and benzoic acid as easily degradable organic compounds. In this study, their method was modified for a slurry system. Sediment slurry system at 50 g (dried sediment weight)/L was amended with 200

mg COD/L of substrates including *p*-cresol, phthalate, ethanol and acetic acid (50 mg COD/L each) to evaluate the general microbial activities of sediments.

Preweighed wet sediment was transferred into 125 mL serum bottles in a helium-filled glove bag and sealed with butyl rubber stoppers. Serum bottles with sediment were moved out from a glove bag and purged with helium gas. The easily degraded organic compounds (200 mg COD/L) containing 50 mg COD/L of ethanol, *p*-cresol, phthalate and acetic acid each and a non-sulfate mineral salts solution (Table 3.3.2.) were added into each serum bottle. Helium was used to exclude O_2 again. The dried sediment weight to media volume ratio in each serum bottle was approximately 50 g/L. Serum bottles were incubated at 25^0C and were agitated daily by shaking for two minutes to eliminate mass transfer limitations.

Gas production of anaerobic active microcosms in sediments was measured with a glass syringe weekly (Figure 3.3.3.). The quantity of gas production was recorded on a cumulative basis and a headspace sample was then withdrawn from each serum bottle with a gas-tight glass syringe and injected into GC-TCD for analyses of gas composition (CH_4 and CO_2). Gas production and methane production were used to indicate the activity of the microorganism community. This information can give a good base reference of general microbial activity under anaerobic environments and was used to establish kinetics of degradation.

Chemical	Concentration (mg/L)	Chemical	Concentration (mg/L)
Mineral Sources		**Phosphate Buffer**	
NH_4Cl	530	KH_2PO_4	270
$CaCl_2.2H_2O$	75	K_2HPO_4	350
$MgCl_2.6H_2O$	100	**Trace Metals**	
$FeCl_2.4H_2O$	20	$ZnCl_2$	0.05
$MnCl_2.4H_2O$	0.5	$CuCl_2$	0.03
H_3BO_3	0.05	$NaMo_4.2H_2O$	0.01
Others		$CoCl_2.6H_2O$	0.5
$NaHCO_3$	1200	$NiCl_2.6H_2O$	0.05
$Na_2S.9H_2O$	500	$NaSeO_3$	0.05

Table 3.3.2. Media solution for Anaerobic Microbial Degradation

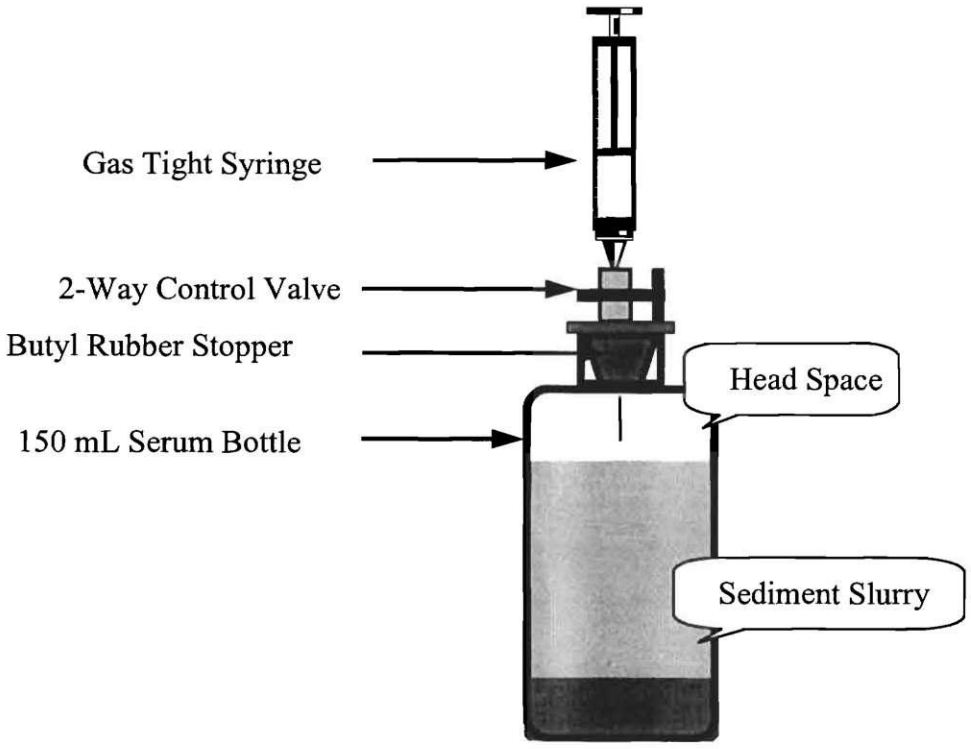


Figure 3.3.3. System configuration of serum bottle

3.4. Aerobic Biodegradation of the COCs

3.4.1. Experimental Procedures

The aerobic respirometric system, as described in Section 3.3.1, was used to measure microbial activity when site COCs were added as substrates. For each sediment, a control slurry containing only the sediment sample and mineral salts was tested for background oxygen exertion. In that sediments had organic matter ranging from 4 to 24 percent (on a total-carbon basis), the need to monitor the biodegradation of sediment organic matter was critical. The experimental slurries were prepared at approximately 50 g/L, based on dry sediment weight and incubated for 60 minutes at 25°C. Duplicate experimental reactors were spiked with 100 mg/L (on an O_2 basis) equivalents of acetone, phenol, benzene, chlorobenzene, 1,4-dichlorobenzene, or TCE. Each contaminant was added to two sediment slurries to monitor experimental reactor duplication and the variation within a specific subsample taken at the same time. Therefore, each COC series includes a sediment blank, a control with easily degraded organics and two replicates with a COC.

3.4.2. Oxygen Exertion Measurements

As in Section 3.3.1, the respirometer was set to monitor oxygen exertion for specific contaminants every 12 minutes with pressure decreases triggering oxygen injection into the system. Outputs of oxygen exertion, injection volume, and injection rates were in spreadsheet format, and the aerobic respirometer data plots showed net oxygen exertion (mg O_2/L) versus time (hours). Each specific contaminant was assessed to qualitatively determine if microbial populations were active and quantitatively assessed to determine rates of substrate utilization.

3.5. Anaerobic Biodegradation of COCs

The anaerobic systems conducted in 50 g/L slurries with additions of both [^{14}C] labeled contaminants (acetone, benzene, chlorobenzene and 1,4-dichlorobenzene) and unlabeled contaminants (acetone, benzene, chlorobenzene, 1,4-dichlorobenzene, phenol and TCE) were used to evaluate intrinsic biodegradation of specific site contaminants. Phenol was not examined in labeled form since biodegradation was established without the need for labeled-carbon mineralization. TCE was unavailable in ^{14}C-form and headspace partitioning of this volatile compound made tracer analysis unrealistic within the scope of this study. The [^{14}C] labeled studies provided data on mineralization ($^{14}CO_2$ production) processes for bioremediation of target site contaminants and the fate of [^{14}C] materials under anaerobic conditions. Unlabeled studies provided data on gas (CH_4 and CO_2) production and fate of the organic contaminants. These tests are run in 10-12

individual serum bottles for each contaminant and are sacrificed over time on an individual basis. These incremental data were used to establish run times which varied between 30 to 60 days. Use of ^{14}C contaminants as markers furthermore minimized the need to be concerned for volatilization as other removal processes competing with biodegradation. This is due to the use of the ^{14}C-label as a marker for confirming whether the labeled compound was mineralized to carbon dioxide regardless of whether it was sorbed to solid surfaces or appeared in headspace pages at some point in the incubation.

3.5.1. Mineralization Studies of Radiolabeled Organic Contaminants

In this study, the target initial [^{14}C] activity was 6,000 (disintegrations/min)/mL, dpm/mL (600,000 dpm/100 mL equals to 0.27 μCi/100 mL; 1 μCi = 2.22* 10^6 dpm), for each organic contaminant in each anaerobic reactor which contained 50 g/L of sediment. Each radiolabeled organic contaminant was spiked into each serum bottle. Additionally, the concentration of added [^{14}C] labeled organic compounds was negligible (on a mass basis) when the final target concentration for site contaminants was 10 mg/L. To reach the target concentration for each serum bottle, 10 mg/L of unlabeled contaminant was therefore added.

The [^{14}C] activity in the supernatant fluid of an undisturbed slurry and in the headspace was measured with a liquid-scintillation system. Two replicates for supernatant fluid samples, one headspace sample and two measurements each in the scintillation system were performed for each serum bottle. Half milliliter of 5N NaOH was added into each serum bottle to dissolve labeled or unlabeled $CO_{2\ (g)}$ into supernatant fluid. One milliliter of supernatant fluid or headspace was withdrawn next and added into 5 mL of scintillation liquid. This measurement was the sum of ^{14}C activity of dissolved site contaminants or any nonvolatile intermediates, volatile intermediates, CO_2, and CH_4.

Ten milliliters of supernatant fluid was transferred into an acidic chamber while the NaOH trap system (Fig. 3.5.1.) was purged with N_2. Glacial acetic acid (0.2 mL) was added immediately and CO_2 was purged out and trapped by NaOH. [^{14}C] in acid chamber and NaOH trap tubes were counted. The counting results for acid chamber represented the [^{14}C]-labeled nonpurgeable and dissolved organic compounds, and the counting results for NaOH trap tubes represented $^{14}CO_2$ produced from mineralization of the contaminant.

After the analyses of supernatant fluid, headspace and $^{14}CO_2$ production, the overall [^{14}C]-labeled materials sorbed on wetland sediment was determined. Serum

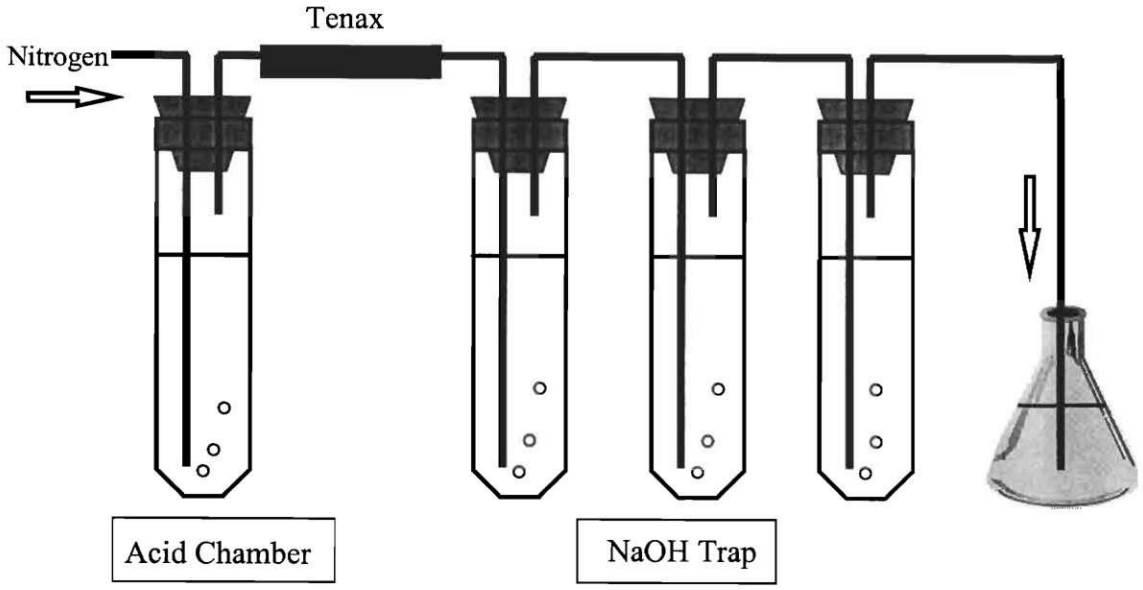


Figure 3.5.1. NaOH trap system

bottle was vigorously shaken and 1 mL of slurry was withdrawn and spiked into 5 mL of scintillation liquid for scintillation counting. The residual slurry was centrifuged and the labeled materials sorbed on wetland sediment were determined by subtracting counting results of centrifugation supernatant from the counting results of slurry.

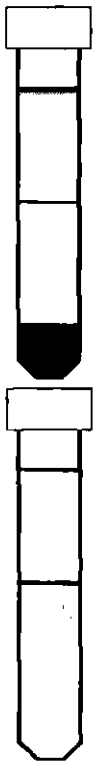
Because the [^{14}C]-labeled materials may dissolve into rubber stoppers, each rubber stopper from serum bottles was shredded and placed in 10 mL of scintillation liquid. This measurement estimated the amount of [^{14}C]-labeled materials dissolved in the rubber stoppers.

3.5.2. Anaerobic Biodegradation of Unlabeled Organic Contaminants

These experiments were conducted in parallel with previous radiolabeled experiments. The method for setup of serum bottles was identical, excluding the addition of radiolabeled organic contaminants. The objectives were to evaluate the production of CH_4 due to utilization of added organic compounds by indigenous bacteria, and distribution of specific site contaminant among supernatant fluid and sediment phases. The setup of serum bottles was consistent with other experiments as mentioned above using a 50 g/L slurry system. Abiotic controls using sterilized sediments were examined as well.

Production of CH_4 was determined by withdrawing headspace samples and injection into GC-TCD. The contaminant retained in the supernatant fluid was recovered using liquid-liquid extraction (Fig. 3.5.2). Fifteen milliliter of supernatant fluid was withdrawn and transferred into a Teflon centrifuge tube, then another 15 milliliter of organic solvent, containing 1 mg/L of bromofluorobenzene (BFB) as an internal standard, was added. Liquid-liquid mixture was vigorously shaken for five minutes to have aqueous sample contact with organic solvent until a homogeneous mixture containing liquid phase and fine pellets was reached. The homogenous mixture was centrifuged at 12,000 rpm for 25 min. at 4^{0}C to breakdown the fine pellets. At this point, the sample was divided into an aqueous phase and an organic solvent phase, and specific contaminants in the aqueous phase were extracted into the organic solvent phase. The extract in the organic phase was transferred into 1.8 mL autosampling vials for GC analysis and the aqueous sample retained inn the centrifuge tube was discarded.

The contaminant sorbed on wetland sediments was estimated using a solid-liquid extraction (Fig. 3.5.2). Ten grams of wet sediment were extracted with 15 mL of organic solvent and 15 mL of methanol. The three phases were formed: a slurry phase, organic-solvent phase and methanol phase. Note that the methanol was added for transferring the water insoluble organic compounds in the aqueous sample into the organic solvent phase and therefore enhance contact opportunities with organic solvent. The three-phase mixture in a Teflon centrifuge tube was vigorously shaken until a homogeneous slurry



Sediment Extraction:

- 10 gram slurry + 10 mL organic solvent +1 ppm BFB + 10 mL Methanol
- Hexane: 1,4-DCB & TCE
  Methylene Chloride: phenol, benzene & chlorobenzene
- Centrifuge:12000 rpm, 25 minutes at 4 C

Supernantant Extraction:

- 15 mL supernantant + 15 mL organic solvent + 1 ppm BFB
- Hexane: 1,4-DCB & TCE
  Methylene Chloride: phenol, benzene & chlorobenzene
- Centrifuge:12000 rpm, 25 minutes at 4 C

Figure 3.5.2. Solvent extraction in Teflon tubes

phase was reached. The homogeneous slurry sample was then centrifuged at 12,000 rpm for 25 min. at 4°C. The centrifuge procedure squeezed out pore water trapped in slurry, pressed the sediment into bottom of the tube, and separated the organic solvent phase, containing the organic extract from the aqueous phase. Note that the aqueous phase now contained the water dissolvable methanol phase as well. The sample extract in organic phase was then transferred into 1.8 mL autosampling vials for GC analysis and the other portions in the centrifuge tube were discarded. The water content of original wet slurry was determined, as well, for mass balance calculations. The extraction samples were stored at 4^0C and were ready for analysis within 2-3 weeks. If preservation is longer than 2-3 weeks, the samples were frozen.

3.6. Kinetic Approach

A kinetic approach was established to predict biodegradation kinetic parameters and to provide Rust personnel with an overall approach for use in incorporation of study data in wetland and groundwater models. The kinetics of biodegradation is of great significance for evaluating the persistence of contaminants in wetland sediments. Information on kinetics is extremely important because it characterizes the concentration levels likely to be present at some future time, and allows assessment of whether the site contaminant will be eliminated before it is transported to a site at which exposure may be critical. (Alexander and Scow, 1989).

In this approach, a fundamental equilibrium transport model was first used to describe the fate of chemicals in a water-soil system. Then the approach was to focus on the description of biodegradation kinetics.

Based on the conservation of mass in a subsurface soil or sediment system,

$$\partial M_T/\partial t = -\partial J_Z/\partial Z + \Omega(Z,t) \qquad (1)$$

M_T is the total mass of chemicals in aqueous phase plus chemical mass sorbed on soil, therefore,

$$M_T = \theta C + \rho_S S \qquad (2)$$

where θ is moisture content in soil, C is aqueous concentration of chemicals, ρ_S is soil density and S is sorbed concentration of chemicals on soil particles.

J_Z is the flux of contaminant flow through the matrix and is equal to the advective and dispersion flows,

$$J_Z = J_a + J_d = D_n\theta(\partial C/\partial Z) - qC \qquad (3)$$

where D_n is dispersion coefficient and q is water flow rate. (Note that the flux of water flow equals zero for static batch reactors, such as used in this research project).

$\Omega(Z,t)$ indicates possible fates of chemicals in water-soil system including biodegradation and chemical reactions. In the case of biodegradation evaluation (herein based on net oxygen exertion or evolution of gas (CO_2 + CH_4) in a closed, mixed reactor $\Omega(Z,t)$) represented the degradation of chemicals by bacterial metabolism.

The study of biodegradation kinetics in soil has been empirical, reflecting the rudimentary level of knowledge about microbial populations and activities in soil. An example of an empirical approach is the power rate model (Alexander and Scow, 1989)

$$\Omega(Z,t) = -k_c C^n \tag{4}$$

where C is substrate concentration, k_c is a rate constant for disappearance, and n is a fitting parameter. The model can be fit to substrate-disappearance curves by varying n and k until a good fit is achieved. The model of biodegradation in this study can be described by a complex zero- to first-order Monod model, which we have simplified to a first-order kinetic relationship in our work. Then,

$$\Omega(Z,t) = -k_c C \tag{5}$$

where n equals to one, and k_c is the kinetic constant parameter presented in the preliminary experimental results with units of 1/time.

Therefore, the equation of conservation of mass can be rewritten as

$$\partial(\theta C+\rho_S S)/\partial t = -\partial/\partial Z(D_n\theta(\partial C/\partial Z)-qC)-k_c C \tag{6}$$

and

$$\theta\partial C/\partial t +\rho_S\partial S/\partial t = -\theta D_n\partial^2 C/\partial Z^2+q\partial C/\partial Z - k_c C \tag{7}$$

For a static batch reactor, this equation can be presented as

$$\theta\partial C/\partial t +\rho_S\partial S/\partial t = -k_c C \tag{8}$$

In this equation, S and k_c are described as below.

S (mg-chemicals/g-sediment) is the concentration of sorbed chemicals on soil particles. S can be measured by conducting an adsorption isotherm study. The relationship of S and C can then be constructed with a coefficient, K_p, that is, $S= K_p C$. K_p is called partition coefficient or distribution coefficient. On the other hand, K_D can be predicted by K_{OC} and f_{OC}, that is, $K_p = K_{OC} f_{OC}$.

The extent to which an organic chemical partitions between the solid and solution phases of a water-saturated or unsaturated soil, or runoff water and sediment, is determined by several physical and chemical properties of both the chemical and the soil (or sediment). In most cases, however, it is possible to express the tendency of a chemical to be adsorbed in terms of a parameter, K_{oc}, which is largely independent of the properties of the soil or sediment. K_{oc} may be thought of as the ratio of the amount of chemical adsorbed per unit weight of organic carbon (oc) in the soil or sediment to the concentration of the chemical in solution as equilibrium:

$$K_{oc} = \frac{\mu g \text{ adsorbed/g organic carbon}}{\mu g/mL \text{ solution}} \quad (9)$$

Values of K_{oc} (in the above units) may range from 1 to 10^7. All of the available methods for estimating K_{oc} involve empirical relationships with some other property of the chemical-water solubility (S) and octanol-water partition coefficient (K_{ow}). The relationships are usually expressed in log-log form:

$$\log K_{oc} = a \log (S, K_{ow}) + b \quad (10)$$

where a and b are constants and varied with organic chemicals.

k_c is the first-order biodegradation rate constant. In preliminary batch studies, the biodegradability of target organic chemicals is determined by the removal of chemicals versus time through the biological/biochemical processes of bacterial metabolism. The method to measure biodegradation of chemicals under aerobic and anaerobic conditions is a little different with the direct measurement method described above. The disappearance of organic chemicals shows that bacteria are active and uptake of the organic chemicals and respiration, or oxygen exertion, becomes an action of active bacteria, while they uptake the organic chemicals as substrate and electron donor.

$$\partial C/\partial t = -k_c C \Leftrightarrow \partial[O_2]/\partial t = k_c[O_2] \quad (11)$$

The concentration of organic chemicals in aerobic batch reactor at time zero and time, t, are measured by COD (chemical oxygen demand) titration. COD_0 and COD_t represent the COD measurement at time zero and time t. For anaerobic serum bottle studies, the first-order biodegradation rate constants are directly obtained from the reduction of organic chemicals with time. For both aerobic and anaerobic studies, half-life of chemical compounds can be calculated by

$$t_{1/2} = 0.693/k_c. \quad (12)$$

The biological system used to evaluate the kinetics of biodegradation was a 5% slurry (i.e., approximately 50g/L). The population of microorganisms facilitating biodegradation of target organic contaminants was proportional to the slurry ratio in

biological systems. The modification of k_c values for 5% slurries may be needed by models at the site. Incorporation of various parameters (such as water content, organic content of sediment, cell growth in natural organic sediments, and bioavailability of sediment systems with adsorption-desorption mechanisms) into the biodegradation model is so far unclear. The current status of modifying biodegradation kinetics is to report the values obtained for 5% slurries herein and to indicate that correction to other suspension levels or soil matrices at higher concentrations (e.g., x%) would be corrected by sediment content such as

$$k_{x\%} = (k_{5\%})\ (x\%)/(5\%) \qquad (13)$$

This kinetic approach will assist us in clearly establishing the methods selected to model soil systems and the kinetics of biodegradation in other systems and models.

4. Experimental Results and Discussion

4.1. Sediment Descriptions, Field Observations and Physicochemical Characteristics of Samples

From an overall perspective, it was apparent from field examination and observations that a true bottom-sediment in the wetlands was generally unavailable except in areas of open water. The vast majority of the wetland is a bog-like environment in which a surface, vegetative layer of plants and soil is suspended atop a water column of approximately a 1-meter depth. This surface vegetative layer is typically of sufficient structure to support a person walking on it, probably because of the complex surface root system, intertwined with extensive trees and small soil islands within the wetland. Initial sampling trips in November 1995 and March 1996 were focused on locating a "peat-soil layer" beneath the wetland. In general, this "peat-soil layer" does not exist as a well-defined consolidated soil in the wetland, despite earlier references to this in site documents. It is indicated here that the peat-soil layer is an apparent artifact of sampling "wetland sediments" from the banks of the wetland. This peat-soil layer was apparently a wetland-derived sediment that was consolidated during backfilling activities on the wetland banks associated with road (e.g., Hannah Road) and runway development.

Based on several extensive site visits, discussions with Base personnel and extensive attempts at obtaining peat soils with hand-auger samplings, the following wetland description was developed. The upper surface of the wetland is a vegetated layer "floating" on a water column that is about 1-meter deep. The sediment solids associated with the underside of this floating layer are referred to throughout as wetland sediments and labeled as SED. These sediments were unconsolidated matter attached to the overlying vegetated layer. Manual collection of these sediments was only possible since they could not be captured with traditional hand augers. The only other layer of significance was an underlying clay layer, generally free of organic matter. This layer was overlaying a sand layer. Therefore, the active wetland sediments were those in the upper 10-30 cm of the wetland and were attached to the vegetated surface cover.

Observations made while collecting samples in April 1996 are included in Table 4.1.1. These data indicate that (i) upper sediment layers of the wetland were associated with marginally-aerobic water; (ii) pH was slightly acidic and (iii) upper sediments contained a wide variety of plant matter.

Two clay samples (CLAY-01, CLAY-08) and one sand sample (SAND-01) and nine sediment (SED-01 to SED-09) samples were collected. The sediments can be characterized as being of one of two general types. That is, sediment samples at Sites 02,

Site ID	Depth (in)	Temperature (°C)	pH	Depth (inch)	Dissolved O_2 (mg O_2/L)	Additional Site Notes:
SED-01	0	14.7	6.1	0	2.70	15 inches of standing water to solid; subsurface permeable with meter stick. Subsurface permeable with meter stick. Gas released upon agitation of sediment, but no evidence of identification. No H2S at surface. Sediment is fibrous.
	7.5	13.8		7.5	1.60	
	15	13.0		15	1.30	
SED-02	0	17.4	5.8	0	3.50	Lawn of duckweed with a filamentous algae and emerging lawn of broad-leaf aquatic plant
	12	16.0	5.0	12	2.00	
	24	14.4	5.5	24	0.60	
SED-03	5	15.0	6.2	10	0.20	Limited standing water compared to March samples.
	15	14.0	6.1	18	0.20	
				24	0.20	
SED-04	0	14.3	6.5			When sites are agitated with shovel, gas is released beneath the surface. Area is previous discharge channel for Wastewater Treatment Plant.
	9	14.2	6.4			
SED-05	5	15.0	4.9	3	0.15	Small surface rivulets, i.e., drainage canals
	12	15.9	5.0	12	0.12	
SED-06	0	13.6		0	6.00	
	18	14.6		18	0.23	
SED-07	0	12.1		0	0.30	
	18	13.1		18	0.15	
SED-08	0	11.7				Heavy straw bed on top. Surface water is high inFe(III) because of ferric hydroxide, indicated by a red fluffy floc. Surface DO is measured at 9.16 and T of 18.1°C. This T is taken in light exposed surface water; reading is higher than that taken under the overlying straw bed (11.7°C). Clays at this site are most "dense" (i.e., more plastic and "sticky").
	18	13.4				
SED-09	3	10.8				Limited standing water until 150ft. into canopy of trees. At 150ft. from road, 1 to 1.5 ft of standing water. Small amounts of organic material visible in standing water.

Table 4.1.1. Table of Field Analysis for NA-RAFB-0496 samples

04 and 09 were obtained as traditional bottom sediments from open waters using a hand auger at the base of an overlying, free-flowing water column (i.e., no root-zone complex). These would be analogous to "typical" stream samples, although they were from a vast wetland system. The remaining were from surface-vegetative layers.

Physicochemical characteristics of NA-RAFB-0496 and NA-RAFB-0996-SED-03 sediment samples are listed in Tables 4.1.2 and 4.1.3. The physicochemical characteristics of sediment samples were divided into two major components: (i) sediment pH, water content, volatility, TC and COD and (ii) pore-water pH, COD, TOC, alkalinity, TDS and major-anion analyses.

Samples designated 02, 03, 05, 06, 07 and 08 were collected from a wetland water column containing three distinct zones: (i) a surface vegetation layer with depth of 10-30 cm; (ii) an unconsolidated sediment layer with sediment mass intertwined within a root-zone complex to a depth of 1+m and (iii) a dense, consolidated clay layer, followed generally by a sand layer. Samples at SITES-02, -03, -05, -06, -07 and -08 were from the unconsolidated sediment layer beneath the root-zone complex or from underlying clay (SITES-01 & -08) and sand (SITE-01) layers. The root-zone samples were obtained by hand-extraction after the root zone was removed from the top layer. These sediments could not be retrieved by a hand auger since they were unconsolidated materials and were suspended as large clumps of soil or sediment.

Sample SITES-01, -04 and -09 were devoid of a surface root-zone, were under an open-surface water column, were consolidated sediments and were generally typical of sediments under a slow-flowing stream or an area of ponded water.

April 1996 samples were used to generally establish the activity of wetland sediments across the wetland on a north-south transect east of Hannah Road (SITES-01, -02, -03, -04, -05, -06) and an east-west transect south of Second Street (SITES-03, -07 & -08). A background sample was collected from SITE-09 at the northern end of the flightline.

As presented in Table 4.1.2 for NA-RAFB-0496 samples, the moisture contents of the clay and sands were generally low (sand at 21.5% and clays at 34-44%). Sediment samples from the stream-like samples (SED-02, SED-04 and SED-09) had moisture contents of 47 to 58 percent, representative in part of the consolidated nature (i.e., collected by hand auger) of the samples and also indicative of a sand fraction associated with these bottom sediments.

Sample Name	pH (1:1)	Moisture (%)		Volatility (%)		TC (mg/g dried sed)		COD (mg/g dry sed)		COD/TC (mg /mg C)
	Measure	Average	Stddev	Average	Stddev	Average	Stddev	Average	Stddev	
SED-01	5.03	58.00	1.15	29.14	0.23	134	2.20	327	10.71	2.43
SED-02	5.31	58.57	1.79	20.03	1.39	94	0.69	215	11.46	2.29
SED-03	5.17	72.85	0.24	23.41	0.59	93	0.82	244	6.74	2.62
SED-04	4.3	49.12	2.83	10.14	1.69	40	0.96	108	13.01	2.67
SED-05	5.24	67.30	1.54	18.46	0.53	71	1.12	193	4.79	2.71
SED-06	5.46	83.33	0.07	35.64	0.27	186	3.34	530	33.67	2.86
SED-07	5.96	82.25	0.05	41.91	0.10	244	5.32	697	71.43	2.86
SED-08	5.57	75.48	0.61	28.01	1.28	144	4.11	369	25.41	2.56
SED-09	5.04	47.44	0.46	9.49	0.19	45	1.38	138	69.32	3.07
SAND-01	4.64	21.51	0.57	2.87	0.21	10	0.42	25	0.80	2.49
CLAY-01	4.49	44.20	1.23	7.64	0.42	9	0.64	20	0.45	2.22
CLAY-08	6.18	34.13	1.72	8.74	0.47	4	0.69	5	0.81	1.17

Table 4.1.2. Physicochemical characteristics of sediment samples of NA-RAFB-0496 sediments (standard deviations are for triplicate analyses)

Sample Name	Moisture (%)		Volatility (%)	
	Average	Stddev	Average	Stddev
SED-03-01	71.91	0.11	22.16	0.43
SED-03-02	79.29	0.08	29.18	0.31
SED-03-03	76.01	0.15	26.14	0.33

Table 4.1.3. Physicochemical characteristics of sediment samples of NA-RAFB-0996-SED-03 sediments (standard deviations are for triplicate analyses)

Sediment samples from under the root-zone complex had moisture contents ranging from 58 (SED-01) to 83.3 (SED-06) percent. These samples were collected manually and these moisture contents are not indicative of the *in situ* moisture content of the sediment/water column from which the samples were obtained; the integrated, water-column moisture content would be much higher. For the samples collected and returned to the lab, the moisture contents (average value of six sediments was 73.2%) are reflective of the high-organic content of the samples and the lack of any significant quantity of sand.

The volatility of a sample was measured as the percent of loss of sample mass upon exposure of a dry (103°C) sample to combustion (550°C) temperatures and was indicative in part of relative organic content. Sand and clay samples had characteristically low volatility values (2.87 - 8.74%), as well as low total carbon (4 - 10 mg/g (or 0.4% to 1% as C)) and low COD (5-25 mg/g (or 0.5 to 2.5% as COD)).

SED-02, SED-04 and SED-09, as traditional sediment samples, had TC values of 40-94 mg/g and COD values of 108-215 mg/g. Wetland sediments SED-01, SED-03, SED-05 and SED-08 had generally higher organic content (TC of 71 - 244 mg/g and COD of 193-697 mg/g). The ratios of COD to TC for the SED samples averaged 2.67 (2.289-3.074), which is the typical value for carbohydrate-like (e.g., $(CH_2O)_n$) material.

SED-03 samples from NA-RAFB-0996 sampling had moisture contents of approximately 75.7% and a volatility of 25.8% and were generally indicative of typical wetland sediments previously collected. TC and COD data were not collected for these samples.

Pore-water data collected from these sediments are included in Tables 4.1.4 and 4.1.5. NA-RAFB-0496 samples had similar pH values (6.03-6.64). The groundwater sample from LF2-18 (GW-LF2-18) had low COD (4.61 mg/L), a trace of ammonia-nitrogen (0.31 mg/L) and low concentrations of sulfate (3.2 mg/L) and chloride (2.52 mg/L) ions.

Sediment pore water was generally high in COD and TOC, indicative of active biological breakdown of sediment organic carbon. TDS levels were also generally high (140 to 237 mg/L), with chloride ion, a non-reactive anion in this system, at relatively consistent levels of 5 - 14 mg/L, as well as ammonia at 1-5 mg/L, a biological nutrient and a product of biodegradation. Nitrate levels were below detection for all sediment samples, as was phosphate. The absence of nitrate, a final electron acceptor for anaerobic respiration of bacteria, is generally indicative of a facultative anaerobic microbial community. Oxygen and nitrate would generally be scavenged by bacteria degrading available organics, followed by the reduction of sulfate to sulfide. Sulfate was below detection in all sediment samples except SED-02, SED-08 and SED-09. SED-02 and SED-09 were typical sediments from stream beds and the presence of sulfate would

indicate a low level of anaerobic activity (i.e., no reduction of sulfate to sulfide). With exception of SED-08 and SED-09, all other sediments had sulfate values below detection, indicative of low redox potential and the likely presence of methanogenic activity.

Sample Name	pH	COD mg-O/L	TOC mg-C/L	COD/TOC mgO/mgC	Alk mg/L as $CaCO_3$	TDS (mg/L)	Ammonia mg-N/L	Nitrate mg-N/L	PO4 mg-P/L	SO4 mg-SO4/L	Cl mg-Cl/L
SED-01	6.03	119.58	39.28	3.04	3.60	227.50	4.74	<1	<1	<1	14.29
SED-02	6.25	176.02	41.73	4.22	1.88	NA	5.18	<1	<1	5.90	7.95
SED-03	6.21	104.48	20.55	5.08	1.61	165.00	0.97	<1	<1	<1	9.29
SED-04	NA	14.26	5.42	2.63	NA	NA	2.02	NA	NA	NA	NA
SED-05	6.64	33.22	10.88	3.05	14.76	236.67	2.93	<1	<1	<1	8.18
SED-06	6.43	48.05	12.95	3.71	5.94	123.33	1.68	<1	<1	<1	5.18
SED-07	6.61	78.05	15.77	4.95	5.34	160.00	1.17	<1	<1	<1	9.76
SED-08	6.46	73.31	17.20	4.26	8.05	140.00	1.73	<1	<1	7.30	10.82
SED-09	6.29	70.02	17.78	3.94	3.12	205.00	2.19	<1	<1	6.89	10.37
GW-LF2-18	6.36	4.61	0.00	0.00	0.00	30.0	0.31	<1	<1	3.20	2.52

NA - not available

Table 4.1.4. Physicochemical characteristics of pore water samples of NA-RAFB-0496 sediments

Sample Name	pH	COD mg-O/L	TOC mg-C/L	COD/TOC MgO/mgC	Alk mg/L as $CaCO_3$	TDS (mg/L)	NH4 mg-N/L	Nitrate mg-N/L	PO4 mg-P/L	SO4 mg-SO4/L	Cl mg-Cl/L
SED-03-01	5.85	146.22	31.29	4.67	10.29	195.30	1.36	<1	<1	<1	8.46
SED-03-02	5.85	216.28	42.37	5.10	9.83	341.80	1.38	<1	<1	<1	6.79
SED-03-03	6.05	192.72	39.48	4.88	14.32	239.30	1.21	<1	<1	<1	6.64
GW-LF2-18	5.50	4.68	0.00	--	5.19	6.86	0.16	<1	<1	<1	1.98

Table 4.1.5. Physicochemical characteristics of pore water samples of NA-RAFB-0996-SED-03 sediments

4.2. General Biological Characteristics

4.2.1. Aerobic MPN

The aerobic Most Probable Number (MPN) is an estimate of the mean density of aerobic microorganisms in a sediment. MPN is reported as an absolute number as estimated by statistical programs, including 95% confidence intervals, in Tables 4.2.1 and 4.2.2.

The MPN for NA-RAFB-0496 samples show differences from sample to sample, with clay samples characterized by low biological activity. SAND-01 was also low compared to other sediment samples, but when comparing the log number for SAND-01 with the log values for other sediments, only 0.5 orders of magnitude separated the sand from sediment samples. High MPN values were seen for SED-09, SED-08, SED-02, and SED-01 with 0.5 to 1.0 orders of magnitude separating these samples from other sediment MPN values.

In comparing April and September samples from the same site, the MPN for 0496-SED-03 samples are typically higher than those of 0996-SED-03 samples. September samples are comparable to the SAND-01 values seen from 0496 samples. Subsamples used for aerobic MPN testing on April samples were stored under aerobic conditions while September samples were stored under anaerobic conditions. The anaerobic storage conditions could affect growth of aerobic microorganisms in the MPN test.

Trends between the absolute MPN and sediment volatility and carbon content were investigated, as shown in Figures 4.2.1 and 4.2.2. SED-09 from the background site had the highest level of aerobic cells ($10^{6.628}$) which could be consistent with the shallow environment from which this sample was taken and representing the sample with the highest potential for aerobic conditions. The remaining SED samples (i.e., SED-01 - SED-08) had the next highest MPN values, in general, and were associated with elevated organic contents. Clay and sand samples generally had low MPN and organic contents. There was no distinct correlation between organic content and MPN activity.

4.2.2 Anaerobic MPN

The population of indigenous anaerobic microorganisms was enumerated under anaerobic conditions. MPN results presented in Table 4.2.3 and Table 4.2.4 show viable bacteria per gram of dried sediment for each sediment sampling location. Sand and clay samples had very low numbers of anaerobic cells, as occurred with the aerobic MPN tests. For the SED samples, SED-09 a background sediment sample had the lowest MPN, unlike the aerobic MPN previously. From the results of sediment physicochemical

Sample Name	MPN: Absolute Number (#/g)			MPN: Log Number (log(#/g))		
	Mean	Lower 95% Confidence	Upper 95% Confidence	Mean	Lower 95% Confidence	Upper 95% Confidence
SED-01	1,098,807	324,764	7,408,524	6.041	5.512	6.870
SED-02	1,125,607	332,685	7,589,220	6.051	5.522	6.880
SED-03	333,904	53,130	803,793	5.524	4.725	5.905
SED-05	475,936	112,833	1,223,997	5.678	5.052	6.088
SED-06	530,318	84,382	1,276,612	5.725	4.926	6.106
SED-07	280,133	79,694	743,283	5.447	4.901	5.871
SED-08	1,922,913	568,338	12,964,917	6.284	5.755	7.113
SED-09	4,243,690	600,317	14,342,767	6.628	5.778	7.157
SAND-01	115,582	18,391	278,236	5.063	4.265	5.444
CLAY-01	39,405	5,575	133,182	4.596	3.746	5.124
CLAY-08	6,992	2,067	47,145	3.845	3.315	4.673

Table 4.2.1. Aerobic MPN number of NA-RAFB-0496 sediment samples

Sample Name	MPN: Absolute Number (#/g)			MPN: Log Number (log(#/g))		
	Mean	Lower 95% Confidence	Upper 95% Confidence	Mean	Lower 95% Confidence	Upper 95% Confidence
SED-03-01	47,385	12,706	113,344	4.676	4.104	5.054
SED-03-02	104,346	26,668	319,015	5.018	4.426	5.504
SED-03-03	99,913	31,758	317,867	5.000	4.502	5.502

Table 4.2.2. Aerobic MPN number of NA-RAFB-0996-SED-03 sediment samples

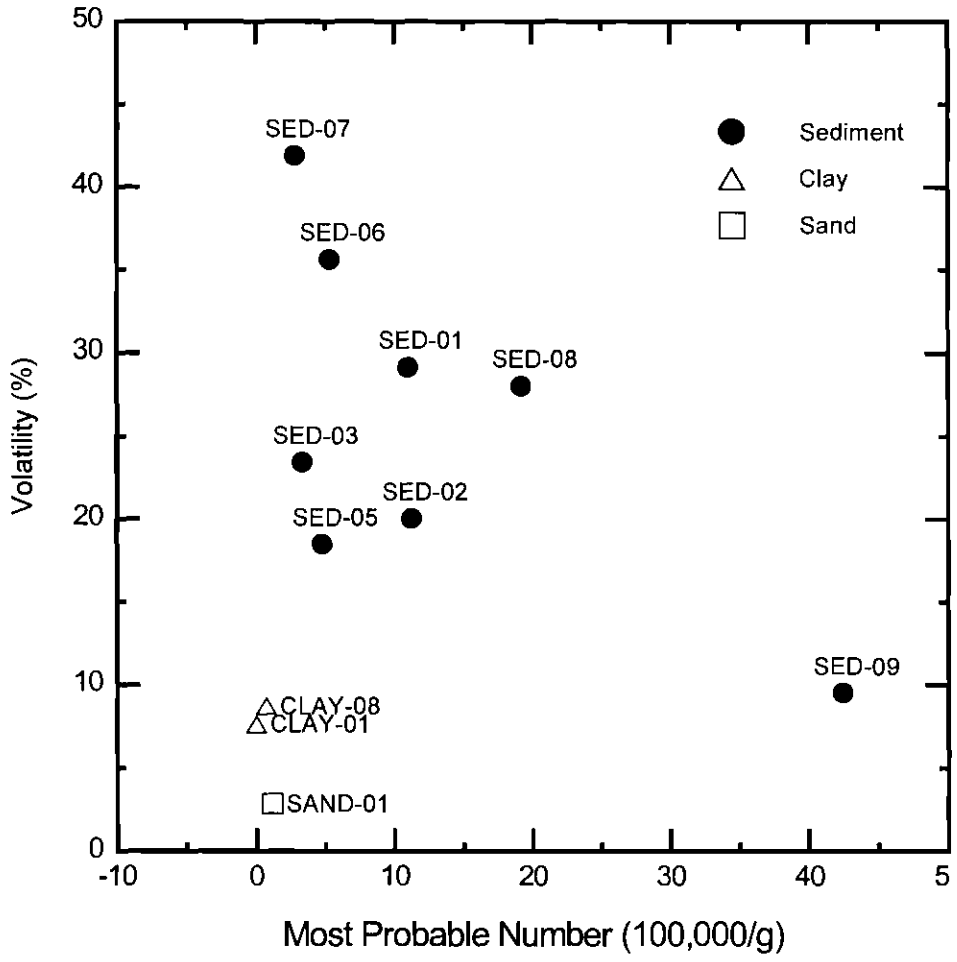


Figure 4.2.1. Relationship between sediment volatility and aerobic MPN of NA-RAFB-0496 sediment samples

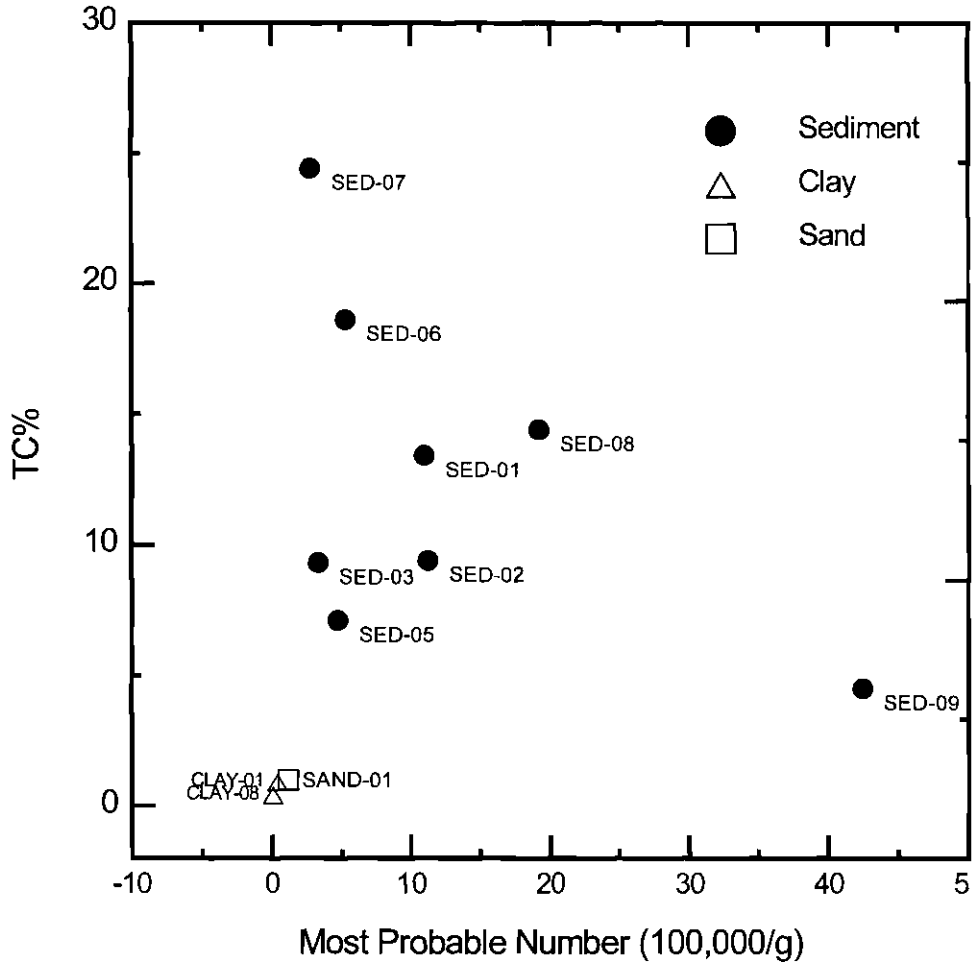


Figure 4.2.2. Relationship between total carbon (TC as %) and aerobic MPN of NA-RAFB-0496 and NA-RAFB-0996 sediment samples.

Sample Name	MPN: Absolute Number (#/g)			MPN: Log Number (log(#/g))		
	Mean	Lower 95% Confidence	Upper 95% Confidence	Mean	Lower 95% Confidence	Upper 95% Confidence
SED-01	120,057	34,155	318,550	5.079	4.533	5.503
SED-02	122,985	34,988	326,320	5.090	4.544	5.514
SED-03	186,756	53,130	495,522	5.271	4.725	5.695
SED-05	373,545	73,433	895,576	5.572	4.866	5.952
SED-06	296,612	84,382	787,006	5.472	4.926	5.896
SED-07	122,594	17,344	414,344	5.088	4.239	5.617
SED-08	91,946	13,008	310,758	4.964	4.114	5.492
SED-09	42,437	6,004	143,427	4.628	3.778	5.157
SAND-01	646	183	1,715	2.810	2.263	3.234
CLAY-01	2,805	664	7,213	3.448	2.822	3.858
CLAY-08	33,435	4,730	113,003	4.524	3.675	5.053

Table 4.2.3. Anaerobic MPN number of NA-RAFB-0496 sediment samples

Sample Name	MPN: Absolute Number (#/g)			MPN: Log Number (log(#/g))		
	Mean	Lower 95% Confidence	Upper 95% Confidence	Mean	Lower 95% Confidence	Upper 95% Confidence
SED-03-01	335,912	100,543	785,952	5.526	5.002	5.895
SED-03-02	1,043,438	266,678	3,190,135	6.018	5.426	6.504
SED-03-03	288,304	92,846	571,754	5.460	4.968	5.757

Table 4.2.4. Anaerobic MPN number of NA-RAFB-0996-SED-03 sediment samples

characteristics of SED-09 had the lowest organic content and total carbon (TC), as well. For other SED samples, MPN ranged from 42,437 to 373,545 per gram, indicative of relatively high cell counts. These values were generally lower than the aerobic MPN values but were similar in number with the exception of SED-08 and SED-09. A comparison of MPN to volatility and carbon content is presented in Figures 4.2.3 and 4.2.4. MPN generally increased with organic content and wetland-sediment samples (SED-01 - SED-08) had higher MPN values, as well as higher levels of organic matter.

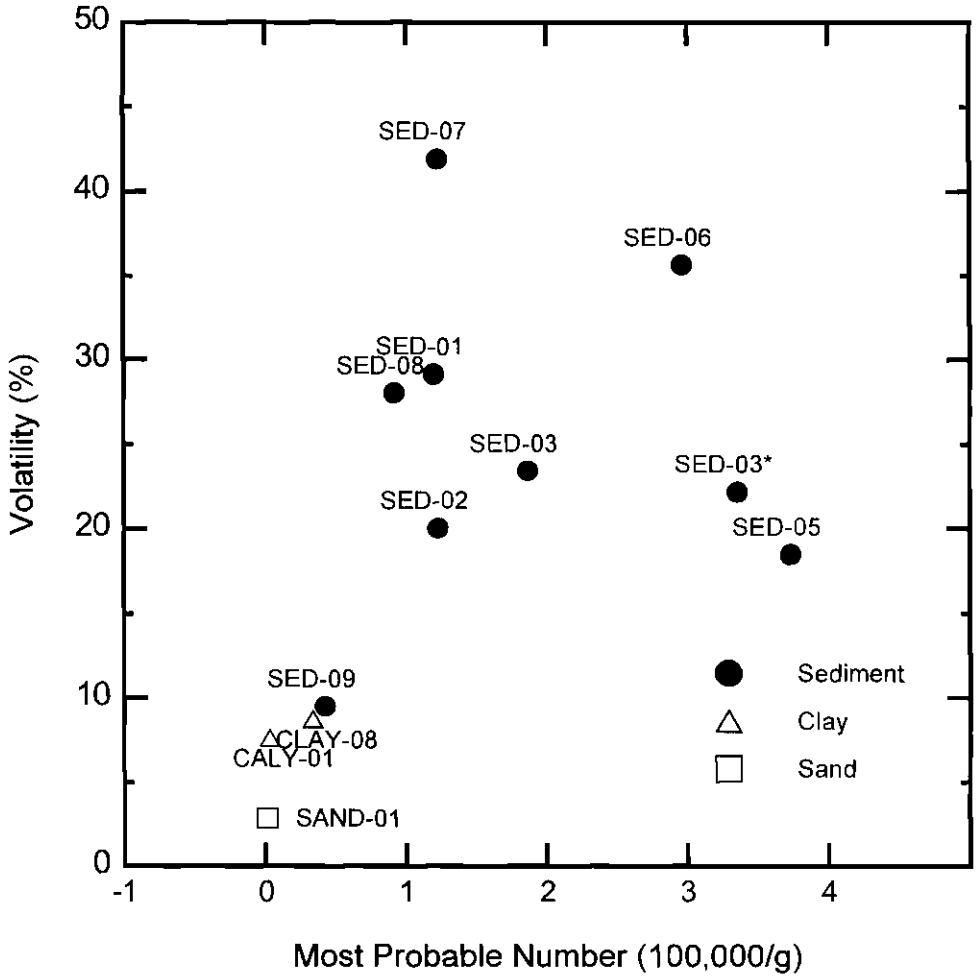


Figure 4.2.3. Relationship between sediment volatility and anaerobic MPN of NA-RAFB-0496 sediment samples.

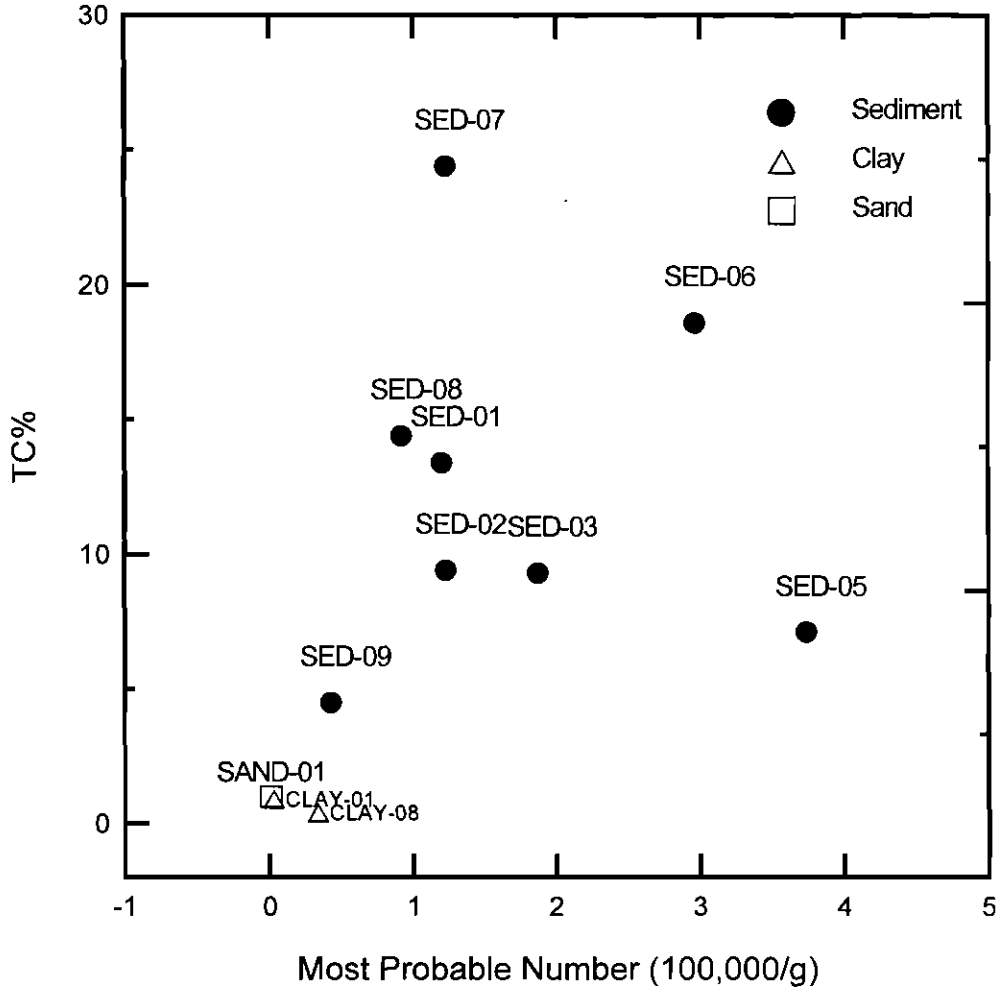


Figure 4.2.4. Relationship between total carbon content (TC as %) and anaerobic MPN of NA-RAFB-0496 and NA-RAFB-0996 sediment samples.

4.3. Microbial Activities Using Easily-Degraded Organic Compounds

4.3.1. Aerobic Studies

The aerobic respirometer data for 0496-SED-01, -03, -08 (denoted SED-01, 03, 08, respectively) and 0996-SED-03 (denoted SED-03*) with addition of easily-degraded organic compounds are shown in Figures 4.3.1. to 4.3.4. Each plot shows the sediment type, the easily-degraded (5 substrate) curves and the background (control) curves. Each five-substrate curve has a corresponding control which was tested during simultaneous experiments. These pairs are shown with corresponding symbols -- the solid symbols with solid lines show the five-substrate data, and the open symbols with dotted lines show the control data.

Controls. SED-01 control curves show varied initial onsets of oxygen exertion. One SED-01 control (triangular open symbol) had initial exponential oxygen exertion during the first 2-3 hours. Another SED-01 control (diamond open symbol) showed initial onset of oxygen exertion at 40 hours. All other controls for SED-01 have oxygen exertion beginning at around 10 hours. After initial onset of oxygen exertion, the controls are linear in shape and increase with a constant slope.

SED-03 controls show a delayed initial onset of oxygen exertion for one replicate (diamond open symbol) and initial exponential oxygen exertion during the first 2-3 hours for all other replicates. After the first five hours of initial onset of oxygen exertion for each replicate, the increase is constant forming a linear control curve.

SED-08 controls show an initial onset of oxygen exertion at times less than 20 hours, with the latest delay at 16 hours (open-star symbol). Two additional replicates (open triangle and open diamond) show delays of 4 to 7 hours, while another two replicates (open circle and open square) show initial exponential oxygen exertion during the first 9-10 hours. After the first 10 hours, the SED-08 controls increase with a linear shaped curve.

All SED-03* controls had delayed initial onsets of oxygen exertion with times of 4, 27, and 45 hours. Two controls (open diamond and open circle) had constant increases after initial onset of oxygen exertion. The remaining control (open star) increases steadily until 58 hours, after which the net oxygen exertion remains constant.

Background oxygen exertion is shown in the control curves since no external organic substrate was added to the reactor. The background oxygen exertion depicted the use of naturally-occurring organic matter as a substrate. This organic matter was in high supply according to volatility, TC and COD measurements but is not readily available for microbial uptake. The sediment organic matter is complex, suspended and colloidal particulates and is not in a soluble, low-molecular-weight form which would be rapidly

consumed by microbial cells. Therefore, the degradation of this sediment organic matter must be preceded by hydrolysis of complex organic compounds to low-molecular-weight compounds with extracellular enzymes. This process is slow, creating a low availability for sediment organic matter. Thus, the system can be described as carbon-limiting or in substrate-starved conditions.

The linear shape of the control curves show that some substrate is made available over time likely due to two processes: endogenous decay of resident microbial cells and the bioavailability of organic matter. During endogenous decay, cell death and lysis allow for the use of cellular components as growth substrates for other viable cells. Naturally occurring organic matter also provides substrate for cell metabolism and respiration. In order for organic matter to be used as substrate, it must be in a soluble form for use by microbes. Both endogenous decay and bioavailability processes depend on enzymatic degradation to produce a soluble form and therefore the linear controls are driven by endogenous respiration and small increments of organic carbon becoming available from hydrolysis of the naturally-occurring organic matter.

The control data for each sediment were further analyzed to focus on active oxygen-uptake phases in Figures 4.3.5 to 4.3.8. Analysis of all controls was performed to eliminate any influence from initial exponential oxygen exertion (e.g., during the first ten hours) and from any initial delays in the onset of oxygen exertion. When comparing replicates, each increased linearly over time at similar rates. In order to compare these replicate controls more closely, linear portions of the control curves were isolated. These portions of the control data eliminate contribution of the initial exponential oxygen exertion during the first ten hours, an increase likely due to (i) oxidation of reduced compounds occurring naturally in the sediments, (ii) acclimation of the microbes to mineral-rich conditions or (iii) acclimation to an aerobic environment. Therefore, the net oxygen exertion after any initial exponential increase was set equal to zero and the axis thus labeled Adjusted Net Oxygen Exertion (mgO_2/L). This analysis of controls also neglected any delay in initial onset of oxygen exertion by setting each replicate's initial onset to a zero time and the axis was thus labeled: Elapsed Time (hours), and appear in Figures 4.3.5 - 4.3.8.

Comparing these sediment control data, SED-08 control replicates had higher values of adjusted net oxygen exertion over the period of study (average 56 mg O_2/L) than SED-01, -03, -03* (47, 26, 27 mg O_2/L, respectively). SED-08 was not considerably different from SED-01, a likely outcome since the carbon percentages in the sediment are similar (14.4% and 13.4%, respectively). SED-03 had a 9.3% carbon content and is less than SED-08 and SED-01, thus providing less background organic matter to be dissolved and made available to microorganisms. However, a combination of microbial content and availability of sediment carbon would control baseline oxygen uptake.

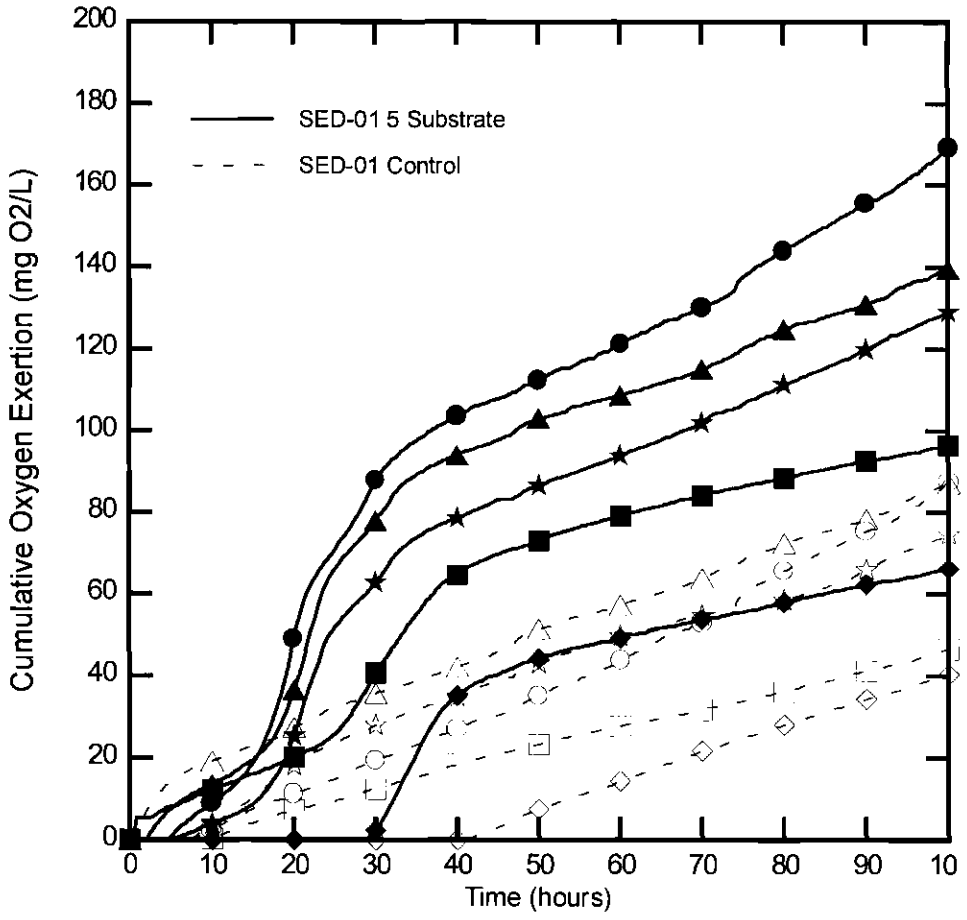


Figure 4.3.1. Aerobic respirometer data for all SED-01 controls and five substrate additions (initial five substrate concentration = 100 mg O_2/L)

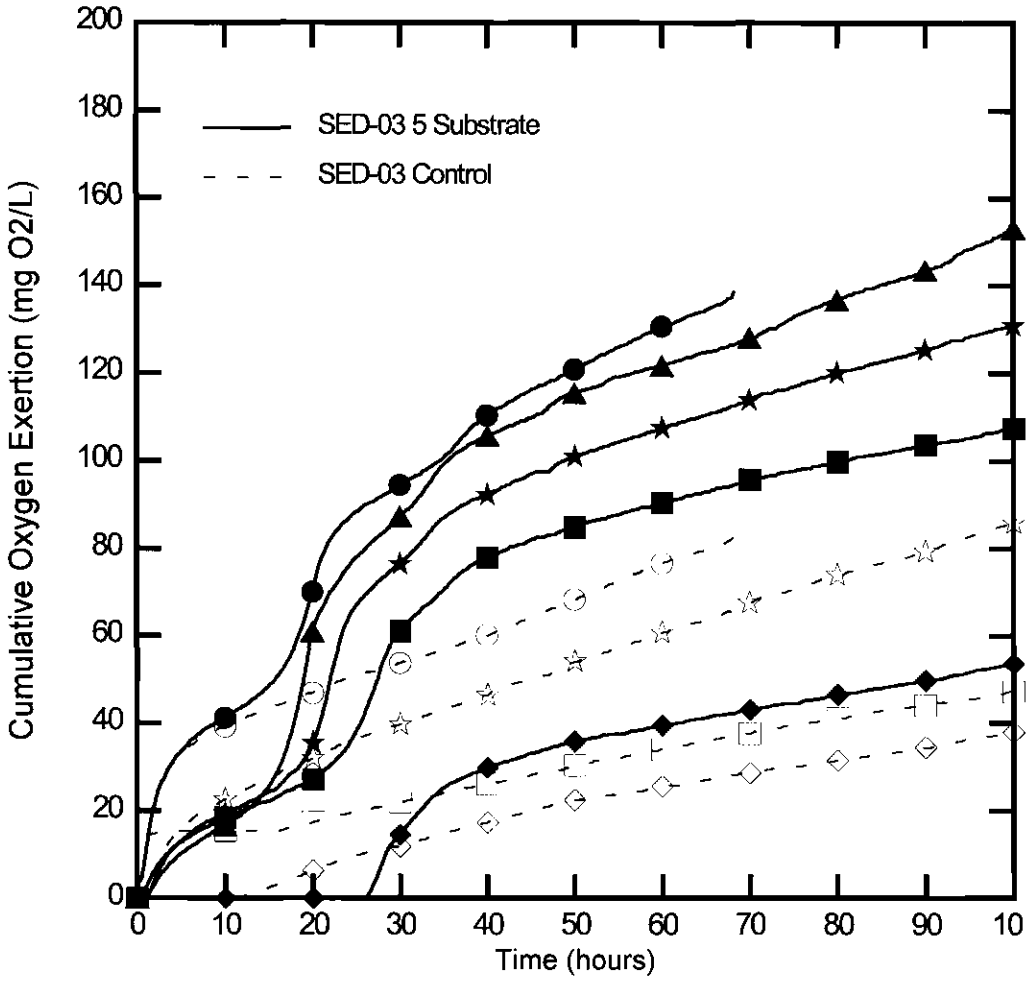


Figure 4.3.2. Aerobic respirometer data for all SED-03 controls and five substrate additions (initial five substrate concentration = 100 mg O_2/L)

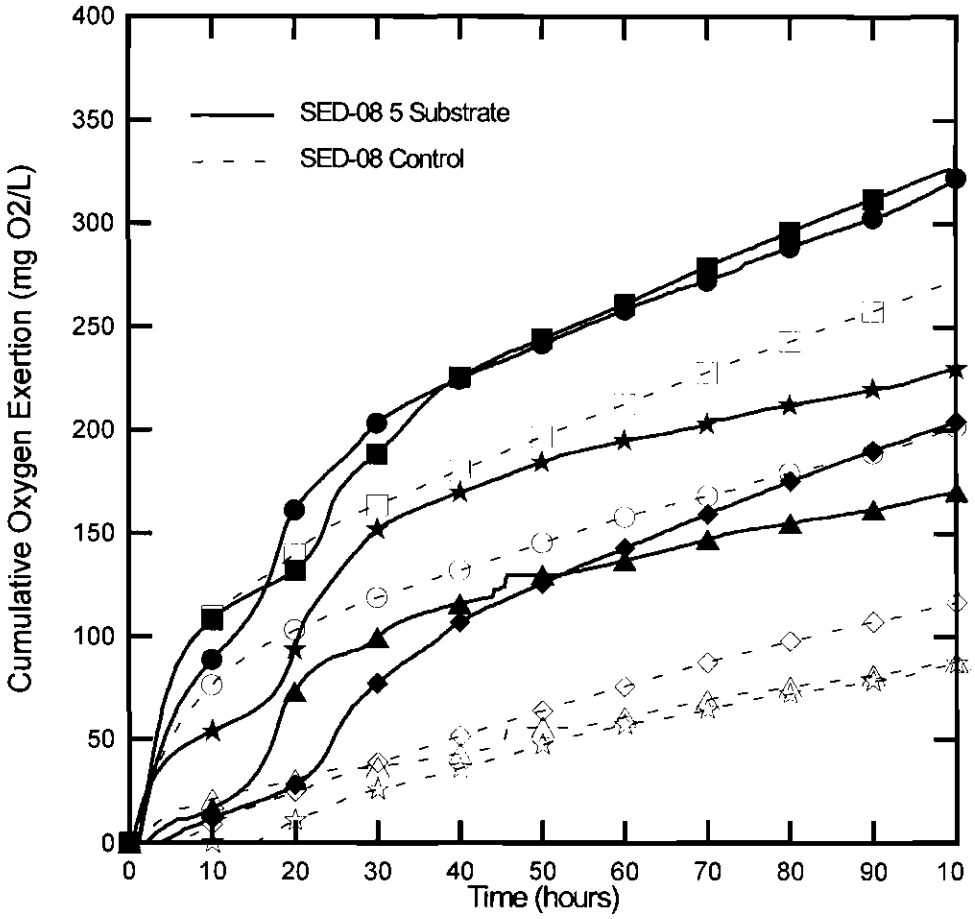


Figure 4.3.3. Aerobic respirometer data for all SED-08 controls and five substrate additions (initial five substrate concentration = 100 mg O_2/L)

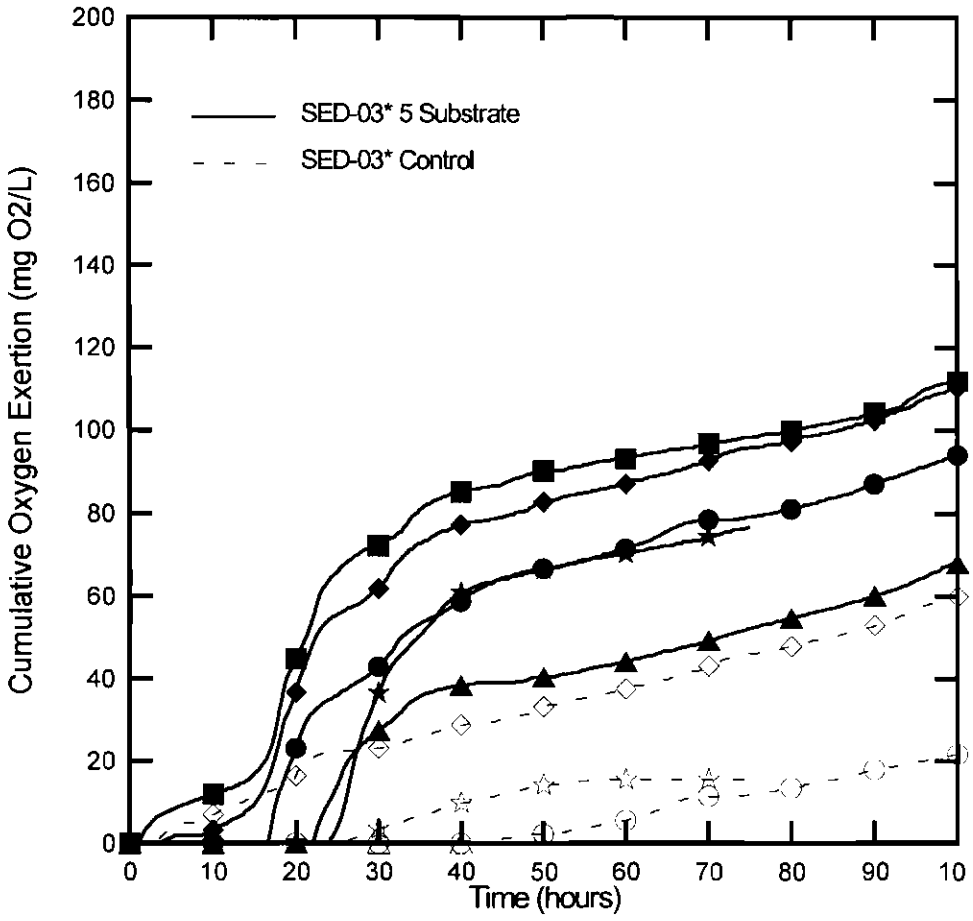


Figure 4.3.4. Aerobic respirometer data for all SED-03* controls and five substrate additions (initial five substrate concentration =100 mg O_2/L)
[* denotes samples of NA-RAFB-0996-SED-03*]

Even though sampled at different times of the year, the adjusted control data for SED-03 and SED-03* were similar. This response appears to signify consistent amounts of background organic material available in the two samples even though collected on different sampling trips.

In summary, these control data indicate that there is a considerable level of biodegradable organic matter associated with the sediments. This organic matter must however be integrated into the overall assessment of organic matter input to the wetlands, in combination with any possible inputs from COCs and other contaminants from Base operable units in surface waters and groundwaters. The natural organic matter being degraded in these sediments furthermore indicates there is a significant input of organic matter to the wetland and this can assist in enhancing degradation of COCs by maintaining an active microbial population and providing primary substrates to facilitate secondary metabolic reactions.

Easily Degraded Organic Substrates. Net oxygen exertion data for SED-01, SED-03, SED-08 and SED-03* are presented in Figures 4.3.9 - 4.3.12. SED-01 five-substrate data show varied initial oxygen exertion times. One replicate (solid diamond) had a delay in initial oxygen exertion of 30 hours. All other replicates have initial oxygen exertion during the first 10 hours. At approximately 15 hours, exponential oxygen uptake began, except for the solid-diamond replicate which began at 30 hours. This exponential oxygen uptake due to the addition of substrate was completed by 40 hours, even though all SED-01 replicates have varied oxygen exertion starting times.

SED-03 five-substrate data also showed varied initial oxygen exertion times. Three of the five replicates were similar in shape during the first 15 hours. The other two replicates varied: one of the two replicates (solid circle) showed exponential oxygen exertion during the first five hours while the other of the two replicates (solid diamond) showed delayed onset of oxygen exertion at 26 hours. For all five-substrate replicates, exponential oxygen exertion was complete before 35 hours. After exponential growth, slopes of five-substrate replicates were similar to corresponding controls (except for the solid triangle which has no control). Unlike other reactors, the open-diamond control had initial onset of oxygen exertion previous to initial onset of oxygen exertion of the five-substrate reactor (closed diamond).

SED-08 five-substrate data show less variation than other sediments in the initial onset of oxygen exertion. All replicates show initial onset of oxygen exertion prior to 5 hours. Three replicates show exponential oxygen exertion during the first 3 hours. This initial exponential oxygen exertion contributes considerably to the final oxygen exertion ending at greater than 300 mgO_2/L. The final oxygen exertion for SED-08 replicates exceed 300 mgO_2/L, while other sediment replicates were less than 200 mgO_2/L. At 100hr, the difference between the five substrate reactors and the control reactors are much greater than the expected 100 mgO_2/L of added substrate.

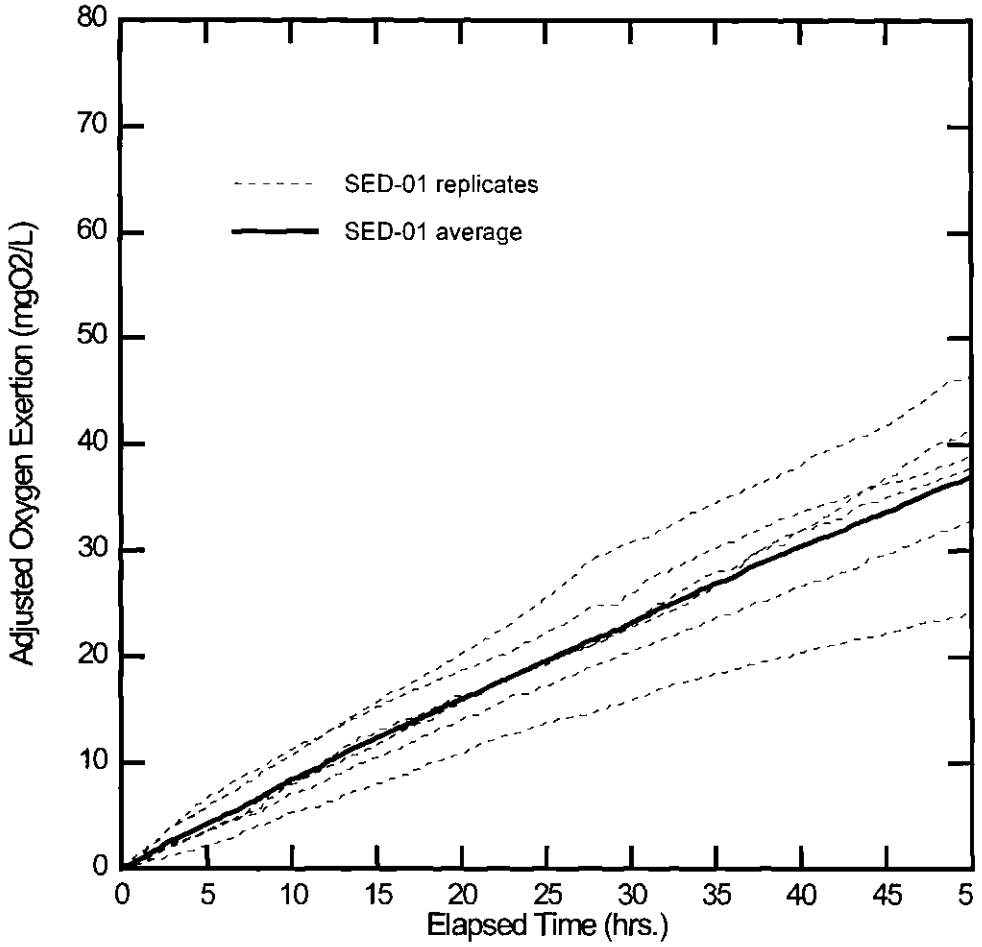


Figure 4.3.5. Analysis of oxygen exertion data for SED-01 controls and adjusted for initial delay in oxygen exertion

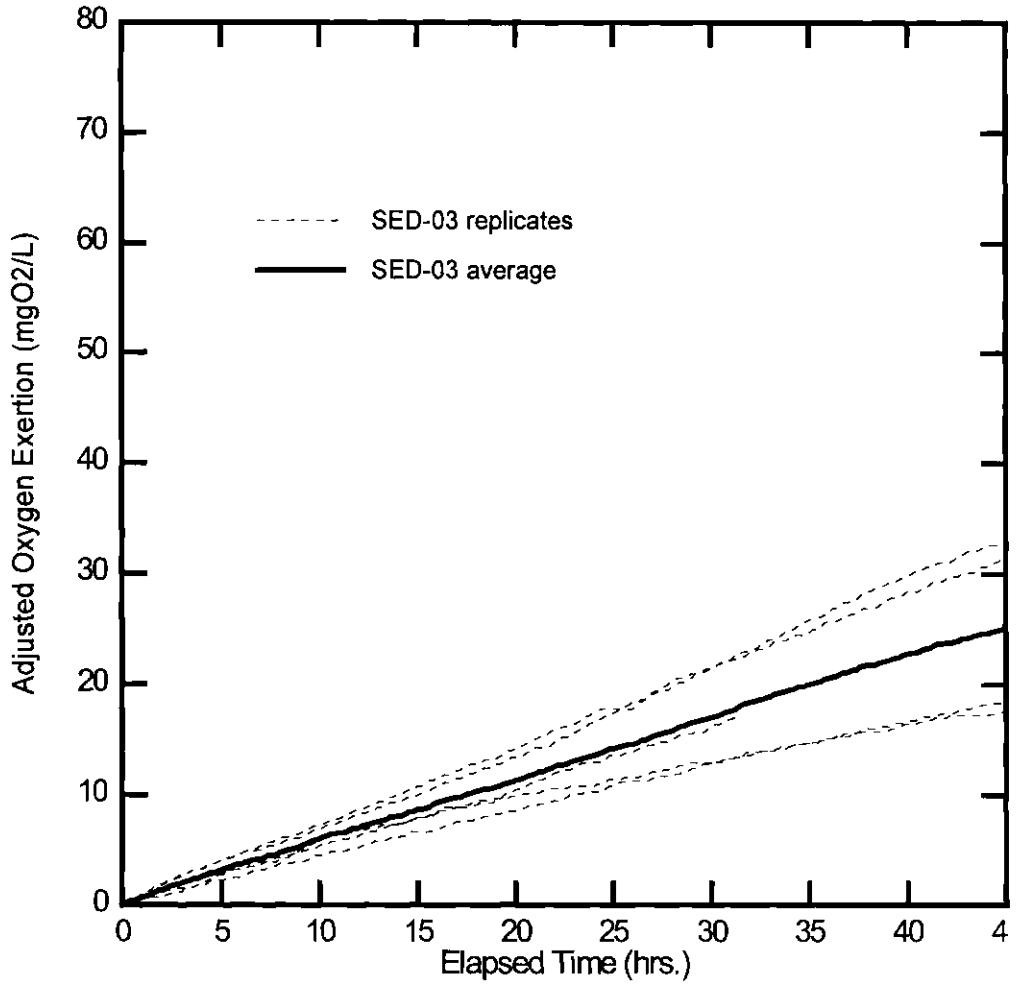


Figure 4.3.6. Analysis of oxygen exertion data for SED-03 controls and adjusted for initial delay in oxygen exertion

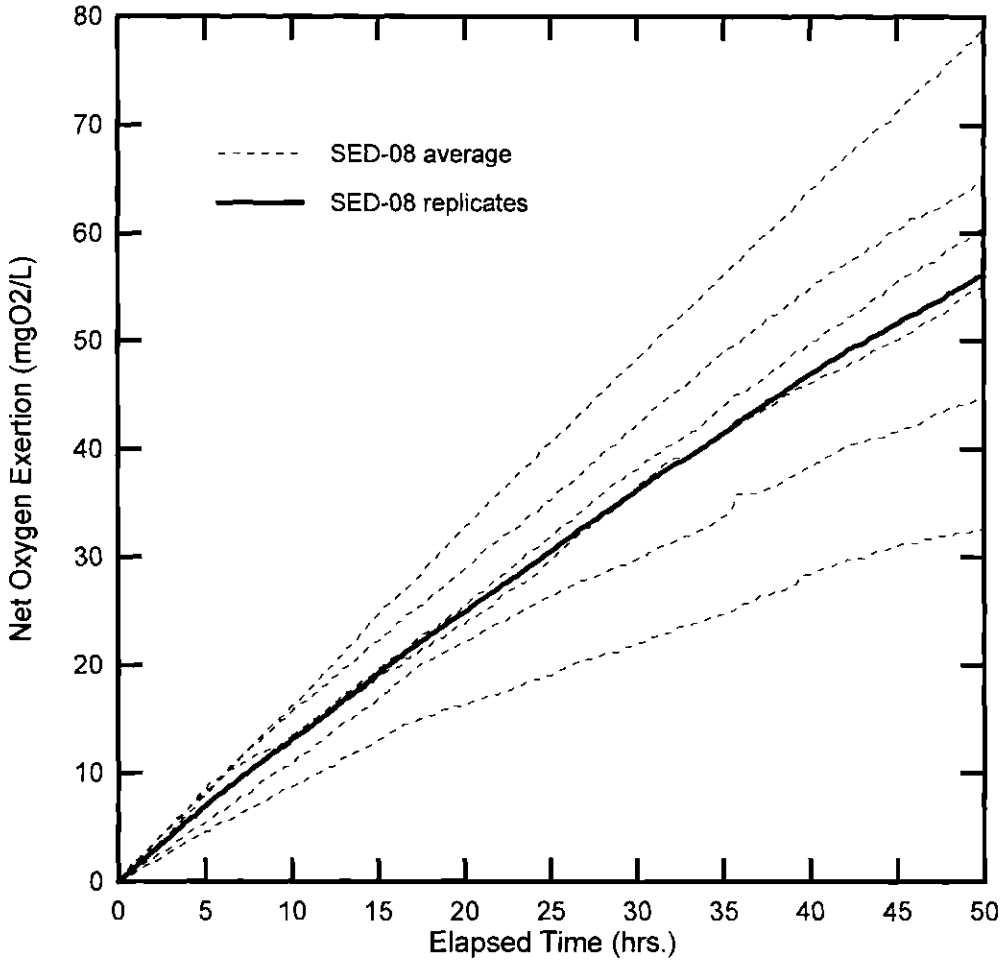


Figure 4.3.7. Analysis of oxygen exertion data for SED-08 controls and adjusted for initial delay in oxygen exertion

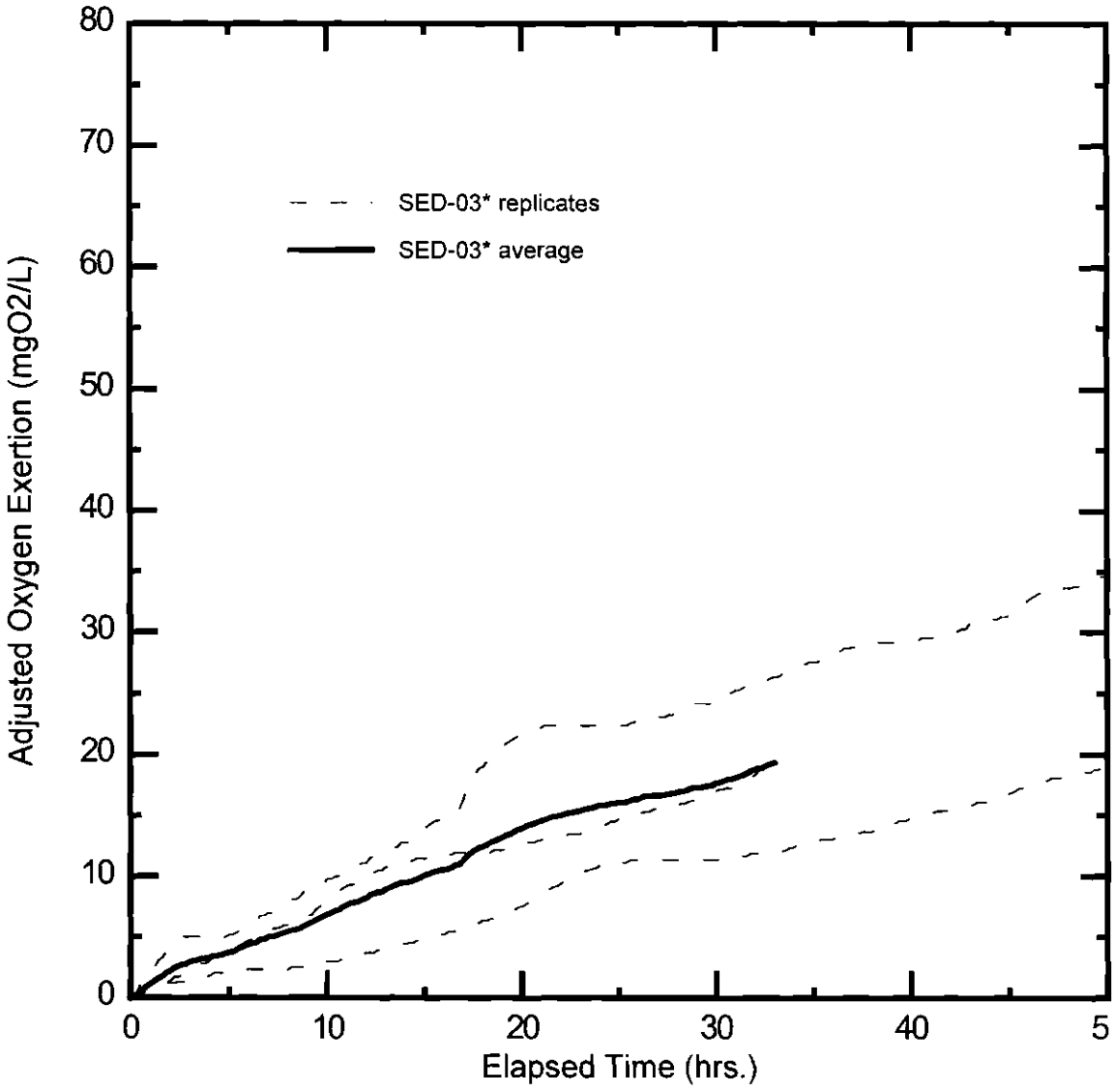


Figure 4.3.8. Analysis of oxygen exertion data for SED-03* controls and adjusted for initial delay in oxygen exertion

SED-03* five-substrate data showed some variance between replicates. The most delayed response for SED-03* replicates was the solid star which initiated oxygen exertion at 24 hours. Two additional replicates showed delayed initial oxygen exertion (solid triangle and solid circle) each with delayed oxygen exertion at 22 and 17 hours, respectively. In general, the final net oxygen exertion of SED-03* replicates was less than other sediments.

When comparing each five-substrate curve with its appropriate control, only the five-substrate curves show exponential increases. Since the added substrate is the only variation between the two reactors, exponential increases are due to microbial responses to the easily-degraded substrates. After exponential increases in the five substrate curves, similar slopes were noted between the control and five-substrate curves. The similar slopes indicate that microorganisms in both systems depended on the background organic matter as a substrate.

The five-substrate data for each sediment were analyzed by focusing on the influence of the added, supplemental substrate. In order to eliminate the influence of the background organic matter, control oxygen exertion was subtracted from the five-substrate data. After control subtraction, any delayed initial onset of oxygen exertion was eliminated for analysis and time was adjusted to represent Elapsed Time (hours). To eliminate the influence of any initial exponential oxygen exertion during the first 0-10 hours, the exponential change during the first ten hours was eliminated and data (Figure 4.3.9 to Figure 4.3.12) are normalized to an Adjusted Net Oxygen Exertion (mgO_2/L).

In observing the analyzed data, there was some variation among the replicates of the same sediment. Because of inherent differences among the sediment subsamples, this was expected. Even though samples were homogenized, changes among the entire sample still occur from one area of the sampling jar, when compared to another area. Over time, compaction of a sample may occur, varying sediment density and thus varying characteristics within a particular subsample. Also, the analyzed data curves are not smooth exponential curves. The curves contain regions of decreases and increases. Since the analyzed data show influences of the substrate only, these are clearly due to the added five substrates. Even though all substrates are easily degraded, some substrates are utilized more readily than others producing small fluctuations in the overall oxygen exertion curves.

In comparing the average oxygen exertion of each sediment, SED-08 responds with the greatest adjusted net oxygen exertion. Other sediments, SED-01, SED-03, and SED-03*, show similar overall responses. Thus, all sediments show aerobic microbial activities for easily-degraded organic compounds.

4.3.2. Kinetic Summary of Aerobic Studies

The kinetic assessment of control data was conducted using a linear regression (SigmaPlot) technique. Zero-order kinetic rates, b_a, and their correlation coefficients (R^2)

are included in Tables 4.3.1 and 4.3.2. The data are identified, within each sediment grouping, as to the target contaminant for which each control was run experimentally.

The zero-order rate constants, b_a, for SED-01 controls ranged from 0.596 to 1.082 mg/(L-hr). The b_a values for SED-03 control ranged from 0.423 to 0.856 mg/(L-hr). Zero-order rate constants for SED-08 are higher than those for SED-01 and SED-03, ranging from 0.86 to 2.206 mg/(L-hr). NA-RAFB-0996-SED-03 (SED-03*) samples ranged from 0.377 to 0.782 mg/(L-hr), within the range of values for SED-03 of 0496 sediments.

As discussed previously, the experimental procedure used in respirometric studies had a targeted sediment concentration of 50g/L (5%) on a dry solids basis. However, the exact sediment concentration in each slurry was unknown until sediment moisture-content calculations were complete (24-72h later). Therefore the sediment concentration, M, did vary. To assess the impacts of sediment concentration variation on the rate constant, zero-order rate constants were normalized to M (i.e., b_a/M). This then allowed for assessment of rates independent of the variation in sediment mass concentration. These data are included in Tables 4.3.3 and 4.3.4 and are expressed in units of mg O_2 per kg of dry sediment per hr (mg (kg·h)). The normalized values for the respective sediments are presented in Table 4.3.5 and ranged from 5.96 - 54.47 mg/(kg·hr) with an overall average for all sediments of 19.34 mg/(kg·hr). It is clear that normalization to sediment concentration was effective in reducing variation in sample rates, and furthermore, this would conceptually allow for prediction of the baseline activity at any sediment solid level.

First-order kinetic analysis of easily-degraded organic compounds produced rate values with units of hr^{-1} and are presented herein as k_{5S}. In general, all sediments reacted with the easily-degraded organic compounds at similar rates with typical k_{5S} values of 0.11 to 0.16 hr^{-1}. All k_{5S} values had low standard errors and coefficients of variance (cv), showing the model fitting well to each data set (Tables 4.3.6 to 4.3.9).

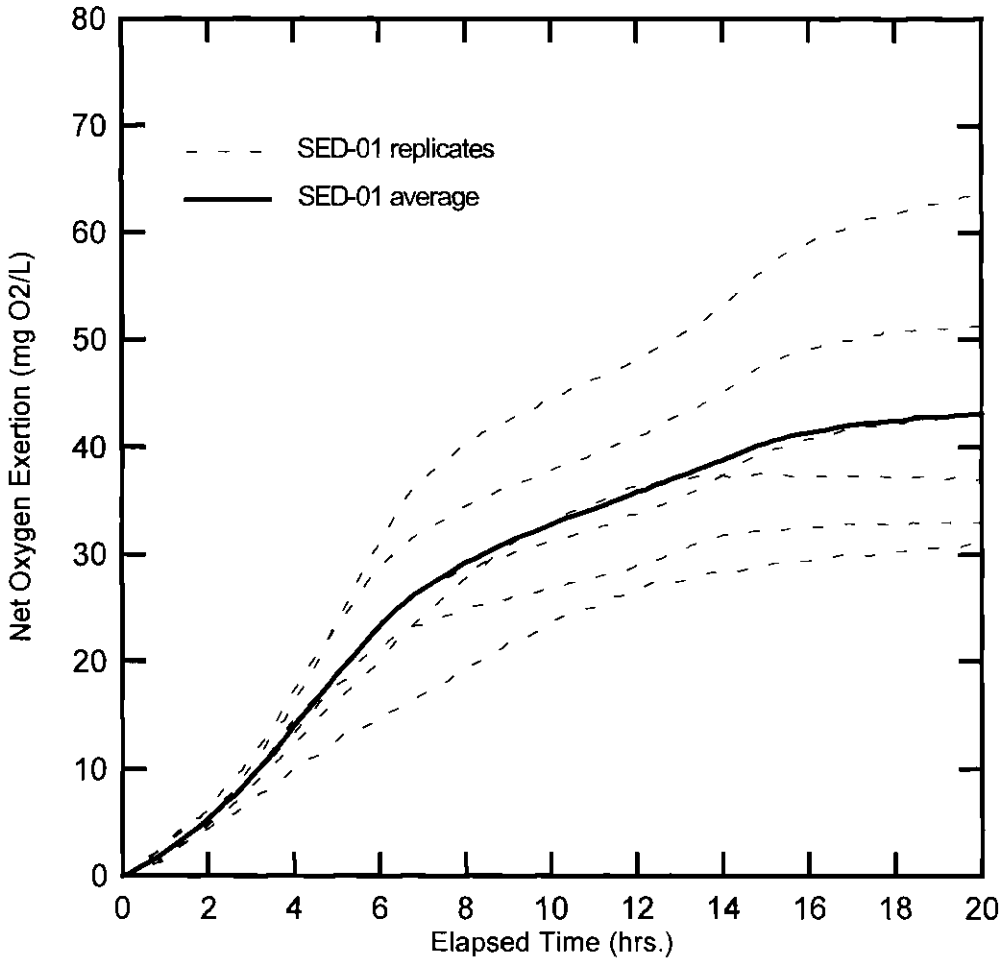


Figure 4.3.9. Analyzed data of SED-01 with control subtraction and lag with five substrate additions (initial COD = 100 mg O_2/L)

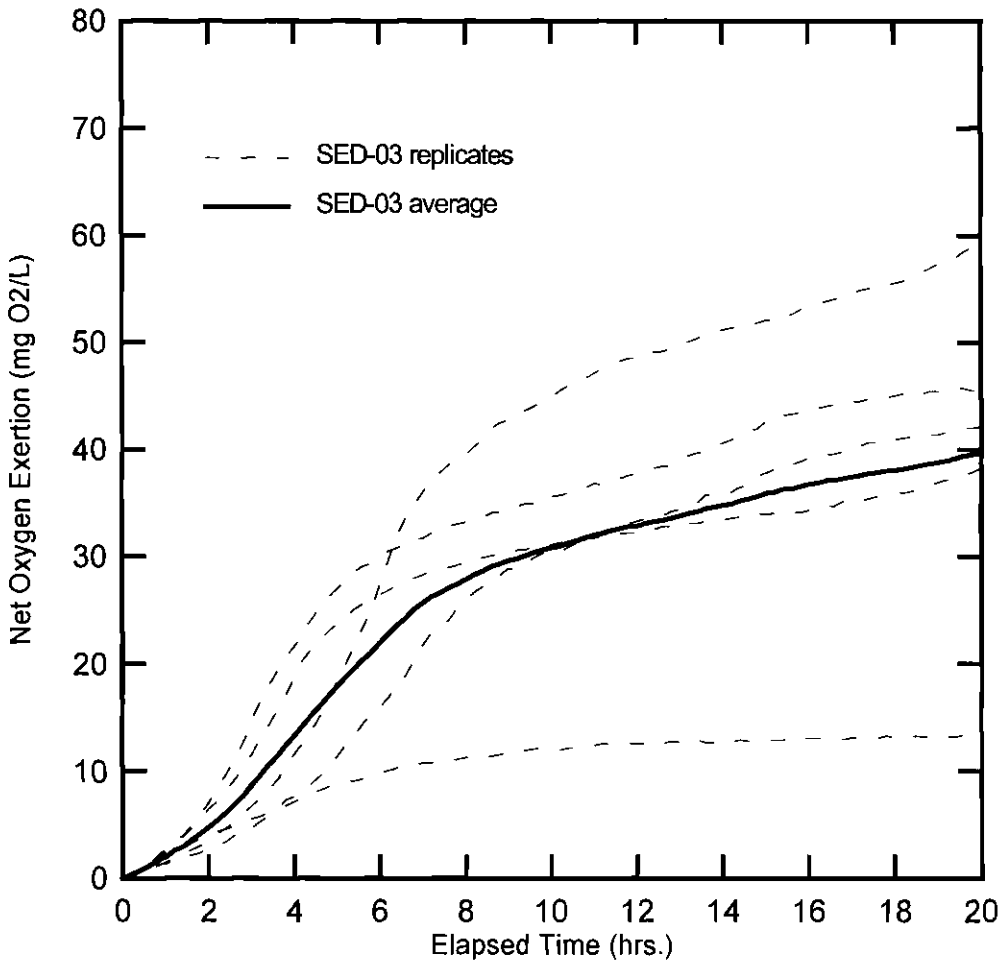


Figure 4.3.10. Analyzed data of SED-03 with control subtraction and lag with five substrate additions (initial COD = 100 mg O_2/L)

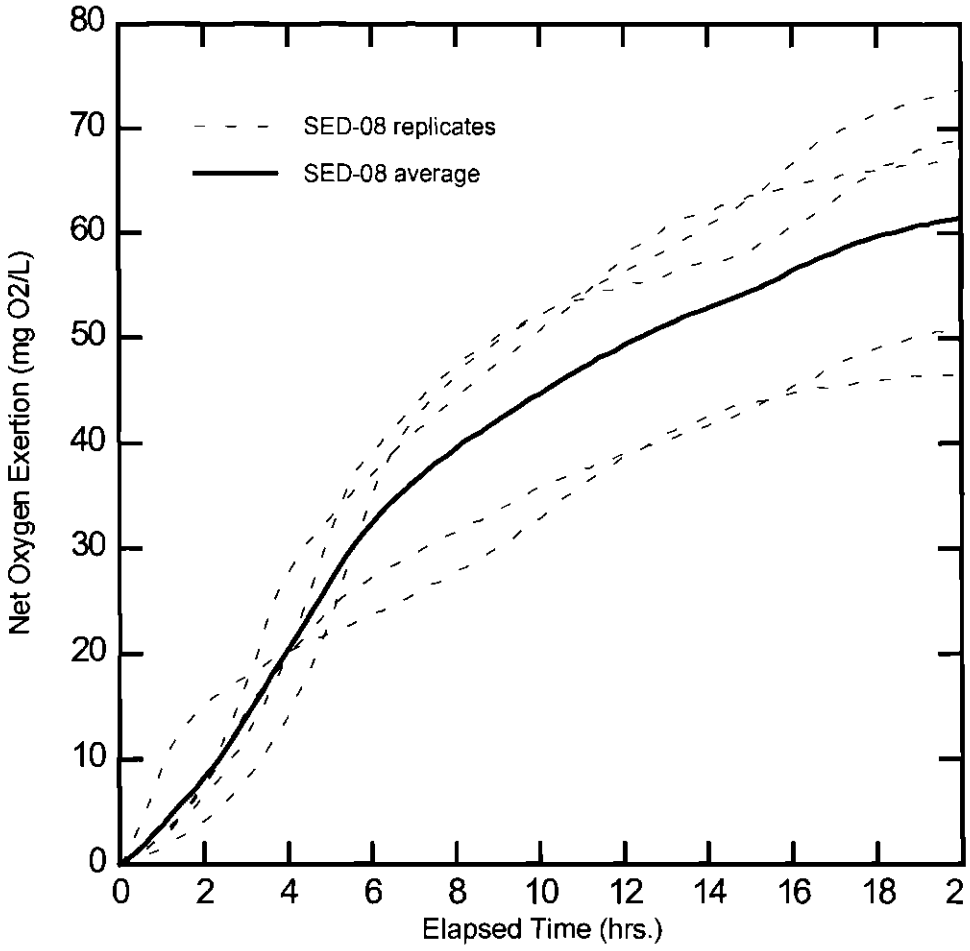


Figure 4.3.11. Analyzed data of SED-08 with control subtraction and lag with five substrate additions (initial COD = 100 mg O_2/L).

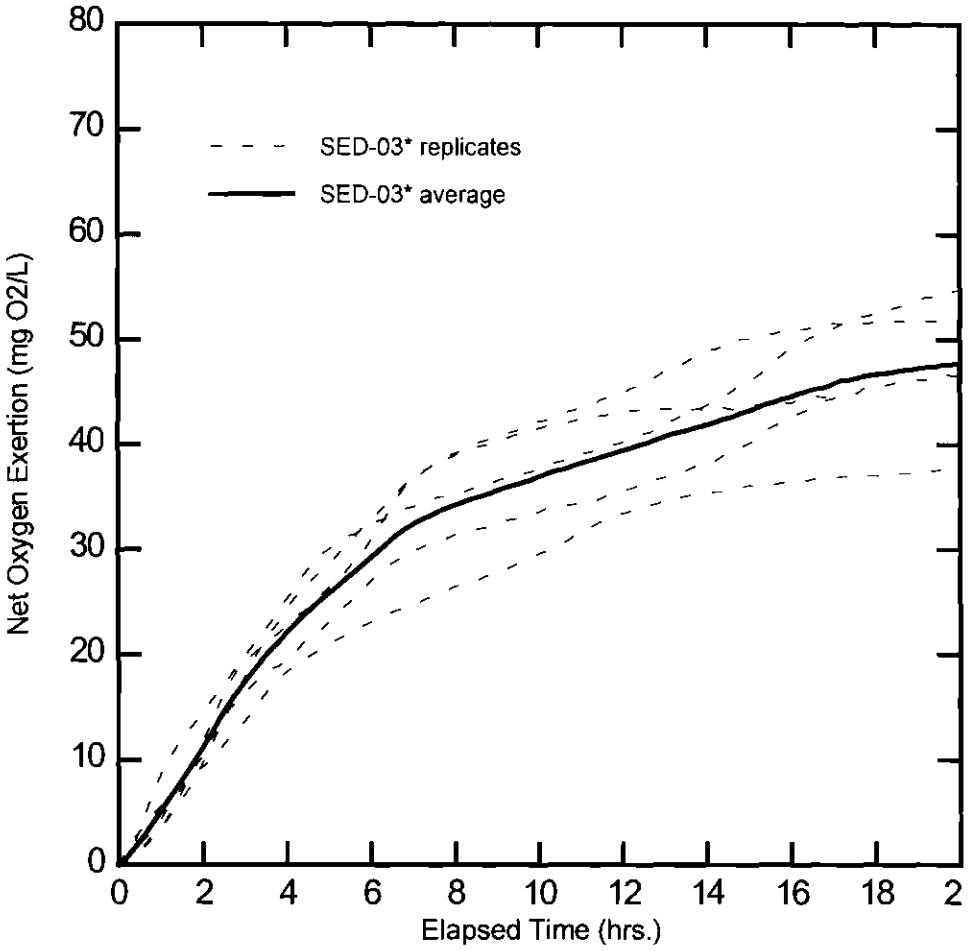


Figure 4.3.12. Analyzed data of SED-03* with control subtraction and lag with five substrate additions (initial COD = 100 mg O_2/L).

Zero Order Model for Controls	b_a	
NA-RAFB-0496 Samples:	(mg/L-hr)	R^2
SED-01:		
Control (acetone)	0.724	0.998
Control (benzene)	0.586	0.992
Control (chlorobenzene)	0.840	0.996
Control (dichlorobenzene)	0.778	0.997
Control (phenol)	1.082	0.990
Control (TCE)	1.063	0.961
SED-03:		
Control (acetone)	0.578	0.987
Control (benzene)	0.423	0.999
Control (chlorobenzene)	0.754	0.997
Control (phenol)	0.500	0.991
Control (TCE)	0.856	0.990
SED-08:		
Control (acetone)	1.498	0.993
Control (benzene)	2.206	0.982
Control (chlorobenzene)	1.736	0.978
Control (dichlorobenzene)	0.860	0.987
Control (phenol)	1.600	0.980
Control (TCE)	1.342	0.983

Table 4.3.1 Kinetic Summary of Aerobic Controls for NA-RAFB-0496

Zero Order Model for Controls	Zero order b_a	
NA-RAFB-0996 Samples:	(mg/L-hr)	R^2
SED-03:		
Control (acetone)	0.782	0.941
Control (chlorobenzene)	0.377	0.983
Control (phenol)	0.586	0.962

Table 4.3.2 Kinetic Summary of Aerobic Controls for NA-RAFB-0996

Normalized k Values NA-RAFB-0496 Samples:	Slurry, M (g_{dry}/L_{media})	b_a/M (mg/kg-hr)
SED-01:		
Control (acetone)	48.425	14.95
Control (benzene)	44.080	13.29
Control (chlorobenzene)	44.018	19.08
Control (dichlorobenzene)	47.327	16.44
Control (phenol)	44.092	24.55
Control (TCE)	29.899	35.55
SED-03:		
Control (acetone)	74.517	7.75
Control (benzene)	70.906	5.96
Control (chlorobenzene)	75.087	10.04
Control (phenol)	72.198	6.93
Control (TCE)	48.763	17.55
SED-08:		
Control (acetone)	100.250	14.94
Control (benzene)	80.233	27.50
Control (chlorobenzene)	77.298	22.46
Control (dichlorobenzene)	47.111	18.25
Control (phenol)	29.374	54.47
Control (TCE)	29.410	45.62

Table 4.3.3 Kinetic Summary of NA-RAFB-0496 Controls Normalized to Sediment Concentration, M.

Normalized b_a Values NA-RAFB-0996 Samples:	Slurry, M (g_{dry}/L_{media})	b_a /M (mg/kg-hr)
SED-03:		
Control (acetone)	66.902	11.68
Control (chlorobenzene)	48.323	7.81
Control (phenol)	48.739	12.02

Table 4.3.4 Kinetic Summary of NA-RAFB-0996 Controls Normalized to Sediment Concentration, M.

Sediments	b_n/M (mg/kg·h)	
	Avg.	Std. Dev.
SED-01	20.64	8.30
SED-03	10.33	5.10
SED-08	30.54	15.93
SED-03*	10.50	2.34
Avg for all sediments	19.34	12.93

Table 4.3.5. Summary of Normalized Oxygen Uptake Ranges for all Sediments

First Order Kinetic Data for 5 substrates	First-Order Rate Constant k_{ss}		
NA-RAFB-0496 Samples:	Value (hr^{-1})	Std Error	CV (%)
SED-01:			
5 Substrate (acetone)	0.16540	0.00573	3.461
5 Substrate (benzene)	0.13130	0.00280	2.135
5 Substrate (CB)	0.11630	0.00242	2.081
5 Substrate (DCB)	0.13440	0.00255	1.896
5 Substrate (phenol)	0.17110	0.00347	2.026
5 Substrate (TCE)	0.13330	0.00246	1.686
SED-03:			
5 Substrate (acetone)	0.18850	0.00144	0.7636
5 Substrate (benzene)	0.11280	0.00336	2.975
5 Substrate (CB)	0.12570	0.00211	1.682
5 Substrate (DCB)	0.10430	0.00200	1.919
5 Substrate (TCE)	0.15740	0.00247	1.571
SED-08:			
5 Substrate (acetone)	0.18850	0.00144	0.7636
5 Substrate (benzene)	0.14100	0.00280	1.986
5 Substrate (CB)	0.11240	0.00295	2.629
5 Substrate (DCB)	0.04284	0.00134	3.138
5 Substrate (phenol)	0.11130	0.00327	2.934
5 Substrate (TCE)	0.12370	0.00112	0.905

Table 4.3.6. Kinetic Summary of Aerobic Microbial Activity Using Easily-Degraded Organic Compounds for NA-RAFB-0496

First Order Kinetic Data for 5 substrates	First Order Rate Constant k_{ss}		
NA-RAFB-0996 Samples:	Value (hr^{-1})	Std Error	CV (%)
SED-03:			
5 Substrates (acetone)	0.11080	0.00128	1.157
5 Substrates (benzene)	0.08835	0.00214	2.420
5 Substrates (CB)	0.09837	0.00121	1.232
5 Substrates (DCB)	0.16290	0.00159	0.946
5 Substrates (phenol)	0.16330	0.00292	1.785

Table 4.3.7. Kinetic Summary of Aerobic Microbial Activity Using Easily-Degraded Organic Compounds for NA-RAFB-0996

Normalized k_{ss}Values NA-RAFB-0496 Samples:	Slurry, M (g_{dry}/L_{media})	k_{ss}/M (L/(kg - hr))
SED-01:		
5 Substrate (acetone)	48.243	3.428
5 Substrate (benzene)	43.994	2.984
5 Substrate (CB)	44.231	2.629
5 Substrate (DCB)	47.249	2.845
5 Substrate (phenol)	44.150	3.875
5 Substrate (TCE)	29.843	4.467
SED-03:		
5 Substrate (acetone)	74.507	2.53
5 Substrate (benzene)	70.997	1.589
5 Substrate (CB)	75.083	1.674
5 Substrate (DCB)	91.972	1.134
5 Substrate (TCE)	48.683	3.233
SED-08:		
5 Substrate (acetone)	100.286	1.88
5 Substrate (benzene)	80.155	1.759
5 Substrate (CB)	77.353	1.453
5 Substrate (DCB)	47.083	0.91
5 Substrate (phenol)	29.390	3.787
5 Substrate (TCE)	29.371	4.212

Table 4.3.8. Kinetic Summary of NA-RAFB-0496 Aerobic Microbial Activity Using Easily-Degraded Organic Compounds Normalized Sediment Concentration, M.

Normalized k_{ss} Values NA-RAFB-0996 Samples:	Slurry, M (g_{dry}/L_{media})	k_{ss}/M (L/(kg - hr))
SED-03:		
5 Substrates (acetone)	66.910	1.656
5 Substrates (benzene)	48.976	1.804
5 Substrates (CB)	48.352	2.034
5 Substrates (DCB)	48.708	3.344
5 Substrates (phenol)	48.776	3.348

Table 4.3.9. Kinetic Summary of NA-RAFB-0996 Aerobic Microbial Activity using Easily-Degraded Organic Compounds Normalized to Sediment Concentration, M.

4.3.3. Anaerobic Studies

Methane production by indigenous microorganisms in NA-RAFB-0496 and NA-RAFB-0996 sediment samples was examined with and without the addition of COCs.

Controls. Methane production for controls without addition of targeted COCs is presented in Figures 4.3.13 and 4.3.14. The cumulative methane produced after 80 to 100 days of incubation varied. Methane production fell within the range of 0.25 to 1.1 mL/g and SED-05 and SED-01 had highest methane production at 1.0 mL/g and 0.75 mL/g after 95 days, respectively. SED-05, however, had the highest microbial population and most active methanogens in all of the NA-RAFB-0496 sediment samples, as shown in Table 4.2.3.

These values of methane production are significant in that they indicate the active methanogenic nature of the sediments and the potential for routine production of methane gas in the wetlands. The overall production rates were collected with 5% slurries but the cumulative methane produced has been normalized to sediment mass so these data can be directly scaled to the wetland sediments. The CH_4-C production rate is significant in terms of carbon flux in the wetlands but is a very small percentage of overall sediment carbon. For example and using a methane production of 0.5 mL/g, this would represent less than ~0.5 percent of the carbon present in a sediment reactor during these studies.

SED-02 sediment samples had no methane production without addition of COCs for 100 days. This result may be explained by the sulfate content in pore water extracted from SED-02 sediment samples, indicating a general lack of indigenous sulfate reducers or the general absence of reducing conditions in these sediments. This sediment was also collected below a 1^+-m water column near a flightline (SAC) apron and was not typical of other locations.

Easily Degraded Compounds. The potential of indigenous microorganisms in sediment samples to degrade a mixture of four easily-degraded organic compounds was assessed to determine general microbial activities under methanogenic conditions (Figures 4.3.15 and 4.3.16). Note that the four compounds were ethanol, cresol, acetic acid and phthlate, with benzoic acid excluded due to solubility limitations. The cumulative methane production from utilization of 200 mg COD/L of these easily-degraded substrates was compared with methane production of sediment controls which had no substrate addition. Net cumulative methane production (Figures 4.3.17 and 4.3.18) was obtained by subtracting methane production of sediment controls from the methane production of sediments with substrate addition. The net methane production values were approximately 1.0 to 1.5 mL/g for all sediment samples collected from various sampling locations.

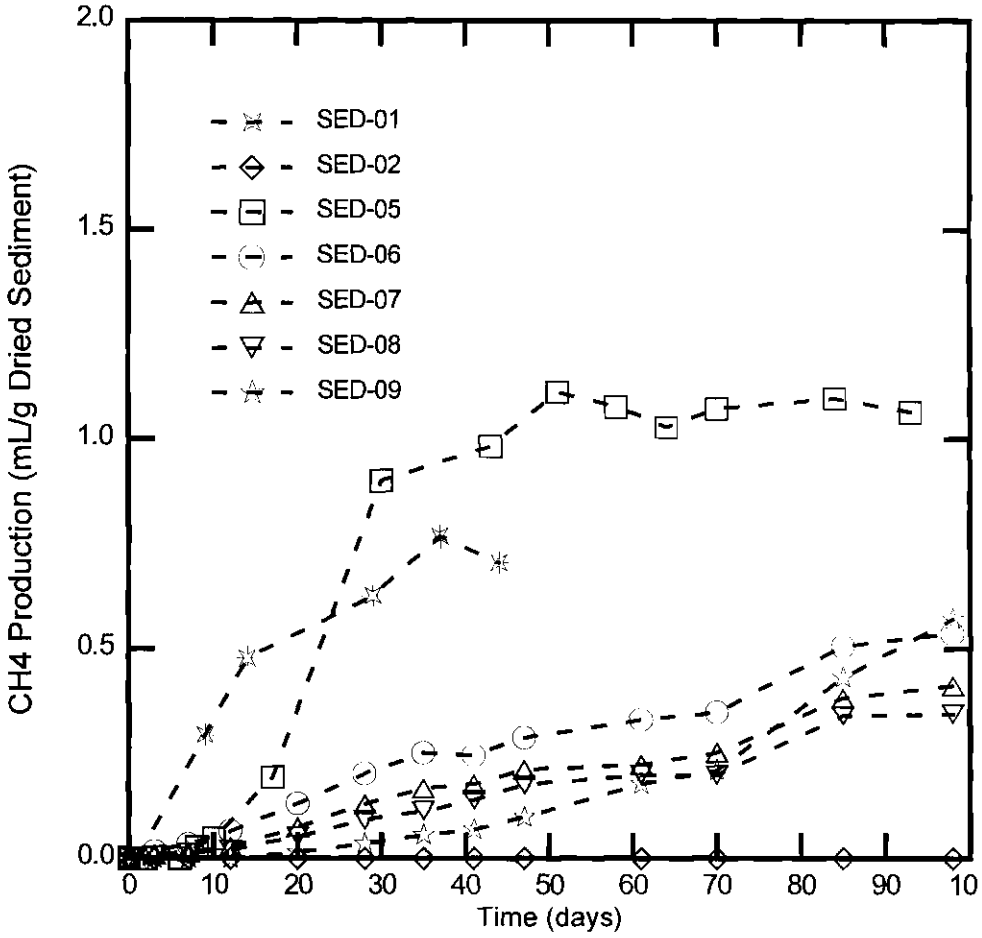


Figure 4.3.13. Cumulative methane production of NA-RAFB-0496 sediments without addition of substrates at sediment concentrations of approximately 50g/L.

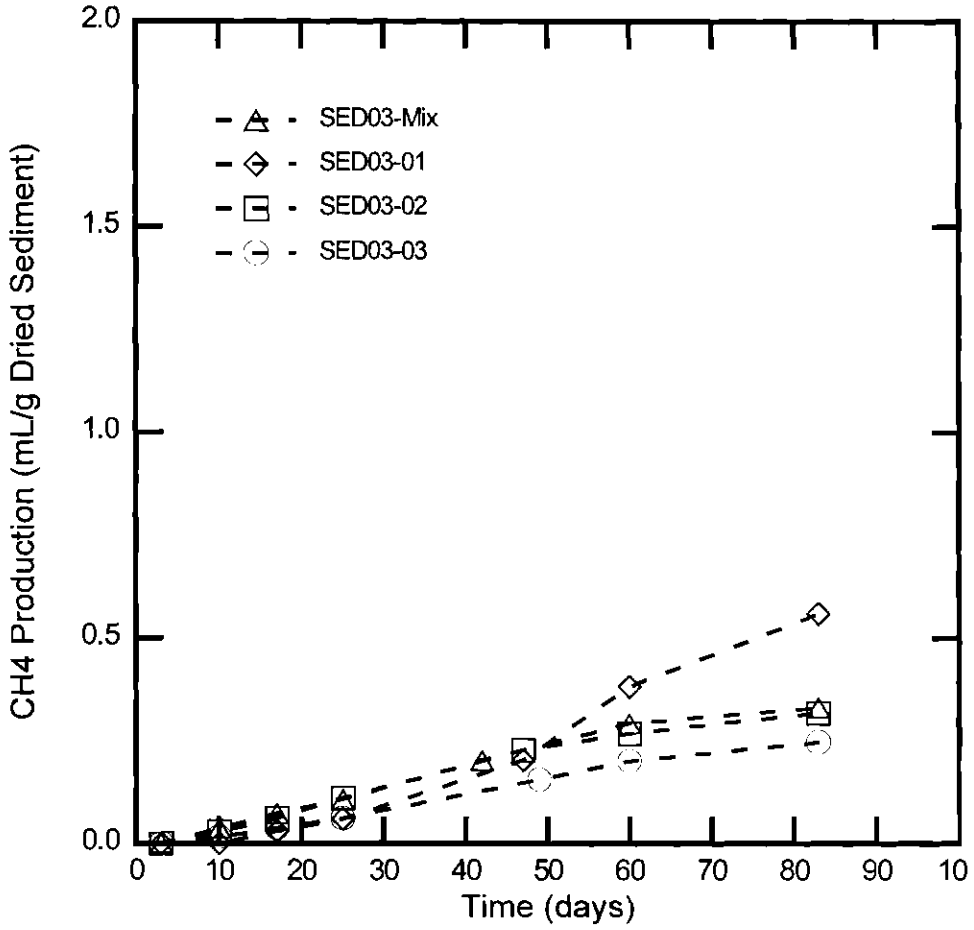


Figure 4.3.14. Cumulative methane production of NA-RAFB-0996-SED-03 sediments without addition of substrates at sediment concentrations of approximately 50g/L.

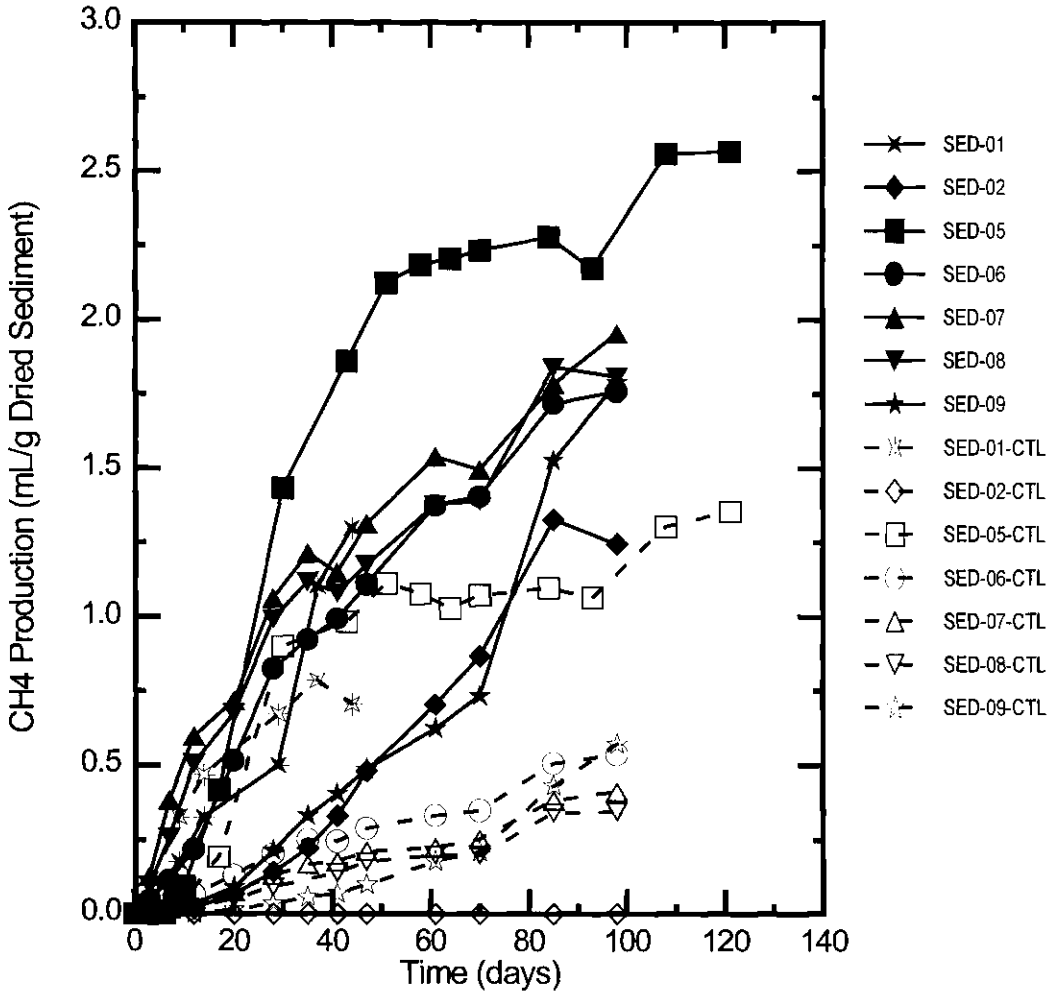


Figure 4.3.15. Cumulative methane productions of NA-RAFB-0496 sediments with and without addition of four easily degraded substrates under methanogenic conditions.

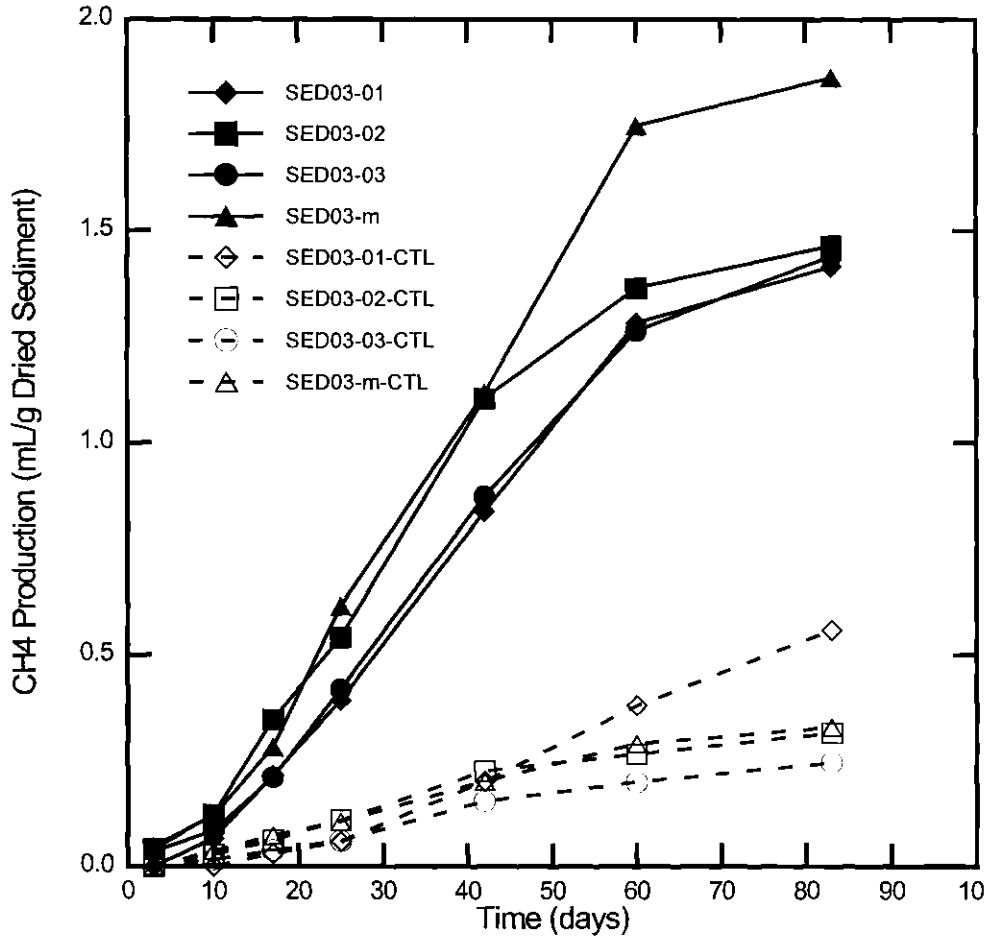


Figure 4.3.16. Cumulative methane production of NA-RAFB-0996-SED-03 sediments with and without addition of four easily degraded substrates.

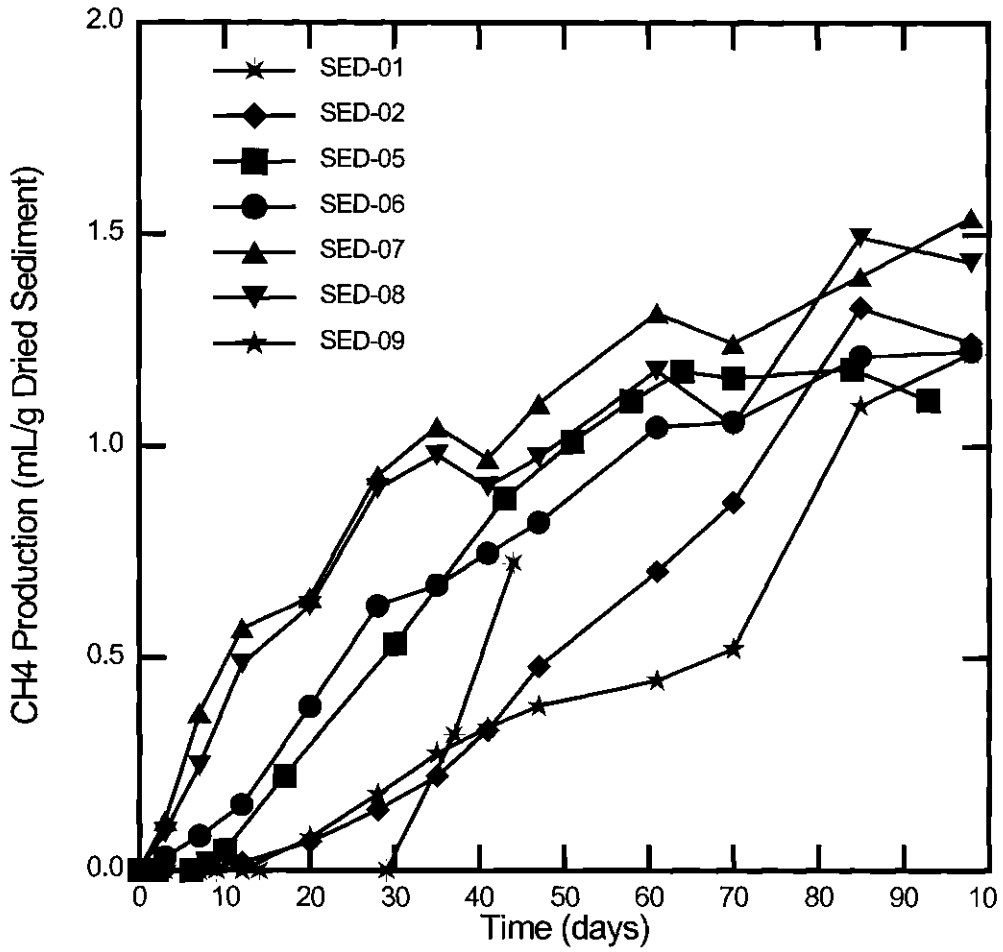


Figure 4.3.17. Net cumulative methane production of NA-RAFB-0496 sediments with addition of four easily degraded substrates under methanogenic conditions.

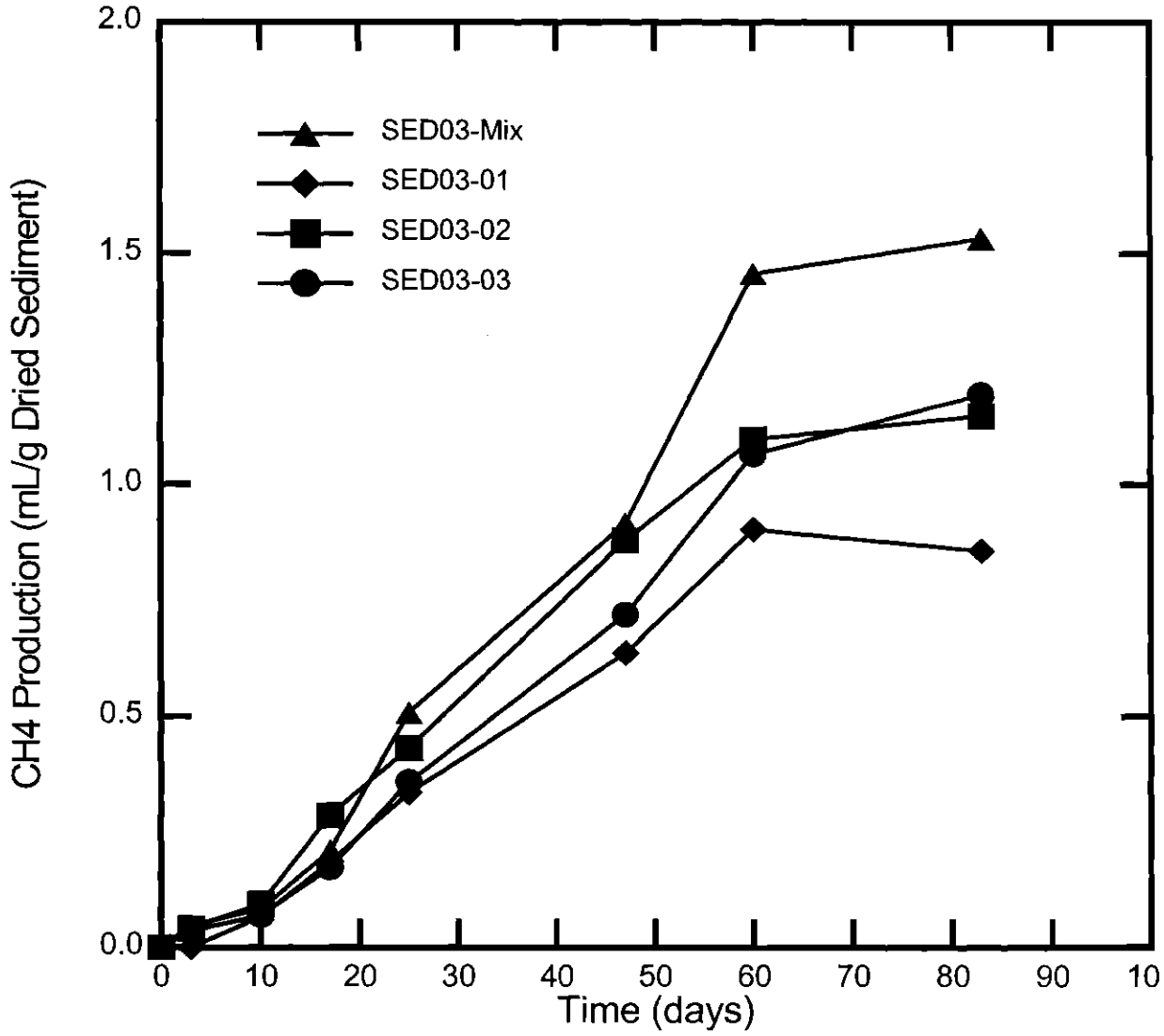


Figure 4.3.18. Net cumulative methane production of NA-RAFB-0996-SED-03 sediments with addition of four easily degraded substrates under methanogenic conditions.

4.3.4. Anaerobic Biodegradation Kinetics of Easily Degraded Compounds

From Figures 4.3.13 and 4.3.14, linear biodegradation of natural organic matter in the sediments was observed for methane production for all sediment samples except SED-02 and SED-05. The data were analyzed using zero-order kinetics and are shown in Table 4.3.10. First-order kinetics of biodegradation in the sediment slurry systems using easily degraded compounds as substrates are illustrated in Table 4.3.11. On the same table, kinetic constants are normalized with the sediment concentration (g/L) to account for concentration effects.

The normalized zero-order kinetic values in Table 4.3.10 show that NA-RAFB-0496-SED-01 had the highest activity. From Figure 4.3.13, it is obvious that SED-05 and SED-06 showed high microbial activity as well. SED-08 and SED-03* (*denotes NA-RAFB-0996 sediment samples at SITE-03) had medium activities for the sediment control and addition of easily degraded compounds. SED-02 had low microbial activity on both conditions. SED-07 has average microbial activity without addition of substrates but behaved poorly when substrates were added. The SED-09 control had low microbial activity (based probably on low sediment organic content); the indigenous microorganisms can however rapidly uptake easily degraded compounds (Table 4.3.11). The extent of degradation of the easily degraded substrates was very high. The substrates added at 200mg/L of COD (total) were equal to approximately 67mg/L of organic carbon. The conversion of maximum net CH_4 production to elemental carbon and normalized to organic carbon added indicated substrate conversions to methane of 48-60 percent for RAFB0496 and 36-60 percent for RAFB0996 sediments.

CONTROL SAMPLES	Slurry, M (g_{dry}/L)	Max. CH_4 Production mL/g	Zero Order b_n Value (mL/kg-hr)
SED-03	45.8	0.329	0.181
SED-01	18.0	0.768	0.181
SED-02	46.1	0.000	--
SED-05	50.0	1.355	0. 574
SED-06	51.1	0.536	0. 237
SED-07	50.5	0.412	0. 173
SED-08	49.3	0.345	0. 144
SED-09	52.8	0.570	0. 168
CLAY-01	53.4	0.000	--
CLAY-08	44.8	0.000	--
SAND-01	60.1	0.000	--

Table 4.3.10. Kinetic summary of methane production of NA-RAFB-0496 and NA-RAFB-0996-SED-03 control sediments at indicated sediment concentrations.

EASILY-DEGRADED SUBSTRATE SAMPLES	Slurry, M (g_{dry}/L)	Max. CH_4 Production mL/g	First Order k Value (hr^{-1})
SED-03	46.0	2.1718	0.00252
SED-01	18.0	1.7475	0.00833
SED-02	47.2	1.3601	0.00257
SED-03	21.0	0.4532	0.00521
SED-05	50.0	2.5677	0.00355
SED-06	50.3	1.766	0.00380
SED-07	51.0	2.342	0.00241
SED-08	48.8	1.795	0.00291
SED-09	51.4	2.263	0.00608
CLAY-01	53.9	0.000	--
CLAY-08	44.6	0.000	--
SAND-01	63.2	0.000	--

Table 4.3.11. Kinetic summary of methane production of NA-RAFB-0496 and NA-RAFB-0996-SED-03 sediment samples with addition of four easily degraded substrates at indicated sediment concentrations.

4.4. Aerobic Biodegradation of Specific Organic Contaminants

Biodegradation of acetone, phenol, benzene, chlorobenzene, and 1,4-dichlorobenzene was observed under aerobic conditions. No oxygen exertion occurred in TCE reactors at amounts greater than that in corresponding controls, suggesting TCE was not biodegradable under aerobic conditions in these sediments.

Like the data for the easily-degraded substrates, the data for specific organic contaminants can be further analyzed by focusing on the net influence of the substrate. In order to eliminate the influence of the background organic matter, control oxygen exertion was subtracted from the specific-contaminant data. After subtraction, any delayed initial onset of oxygen exertion was eliminated and time was adjusted to represent Elapsed Time (hours). To eliminate the influence of any initial exponential oxygen exertion during the first 0-10 hours, the exponential change during the first ten hours was also eliminated.

4.4.1. Acetone

Acetone is commonly used as a solvent for paint removal and is a 3-carbon ketone, similar to propionic acid. Both can be considered to be easily-degraded organic compounds used to assess the general biodegradability of sediments. Acetone can be readily degraded and the results show SED-01, SED-03, SED-08, and SED-03* readily used acetone as a growth substrate (Figures 4.4.1 and 4.4.2). Kinetic data for these sediments are represented in Tables 4.4.1 and 4.4.2 and indicate rate constants, k_c, ranged from 0.048 to 0.16 hr^{-1}.

4.4.2. Phenol

SED-01, SED-03, SED-08, and SED-03* show oxygen exertion when phenol is added as substrate, with little delay in the initial onset of oxygen exertion as shown in Figure 4.4.3. The analyzed data (Figure 4.4.4) also show the relatively fast rates of phenol utilization which is also represented by high k_a values of 0.13 to 0.28hr^{-1} (Tables 4.4.1 and 4.4.2).

4.4.3. Benzene

Benzene was effectively utilized by SED-01, SED-03, SED-08, and SED-03* as shown in Figures 4.4.5 and 4.4.6. The net oxygen exertion due to benzene addition was less than observed for acetone and phenol, thus benzene was not as readily utilized at the same levels of acetone and phenol.

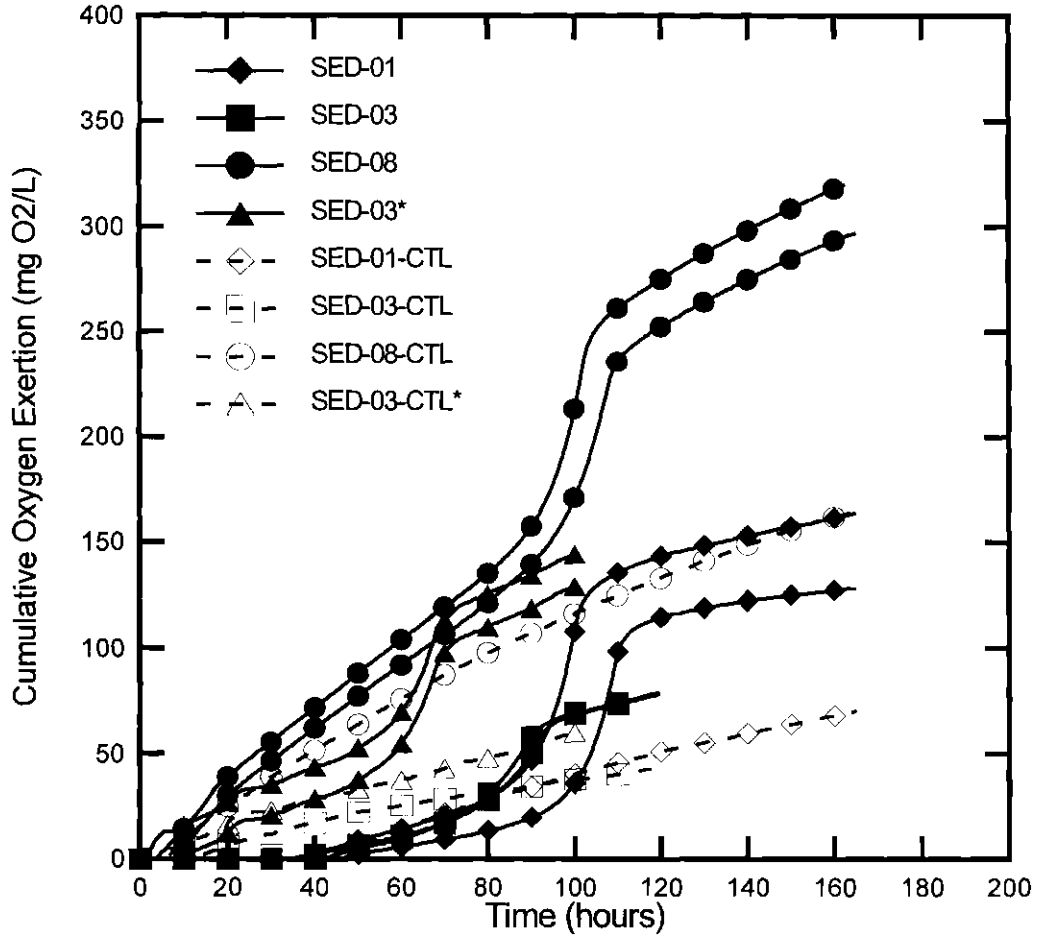


Figure 4.4.1. Aerobic respirometer data for acetone (100 mg O_2/L) addition and control at sediment concentrations of: SED-01, 48.2 g/L; SED-03, 74.5 g/L; SED-08, 100.1 g/L; SED-03*, 66.9 g/L (SED-03* denotes samples of NA-RAFB-0996-SED-03)

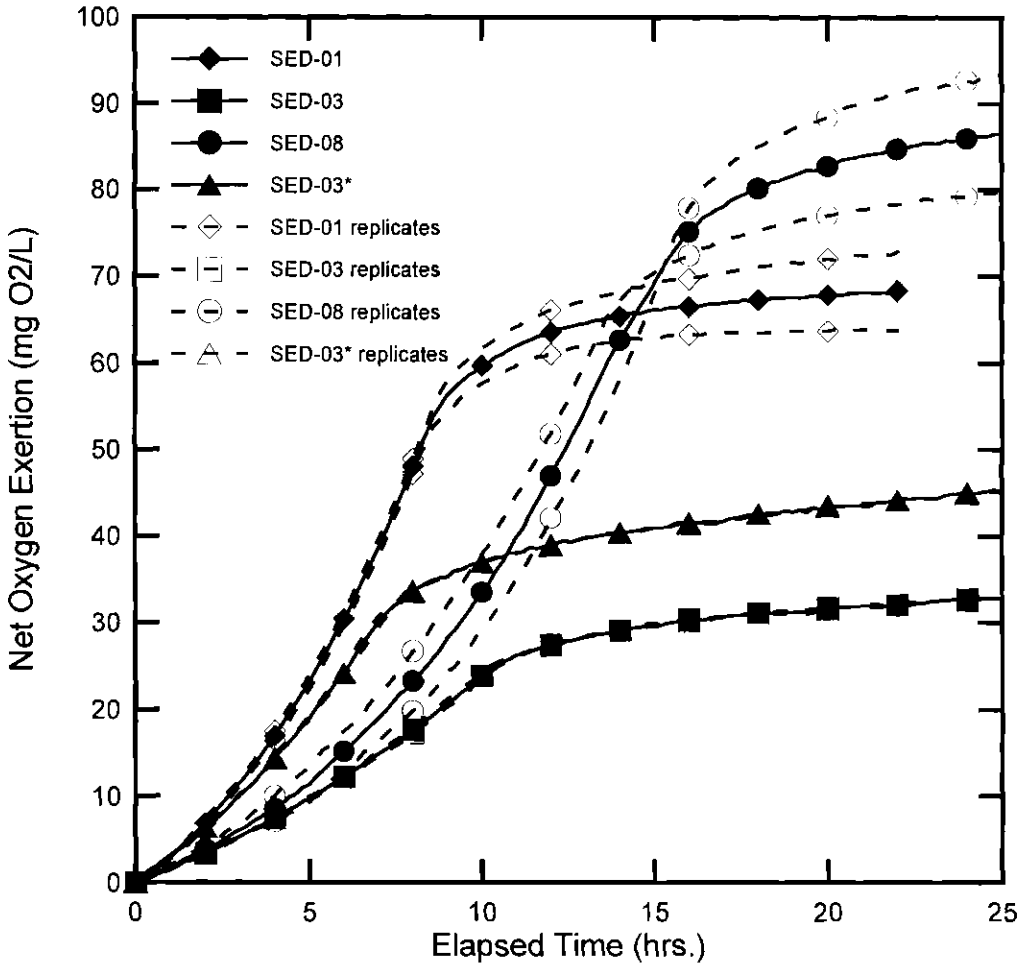


Figure 4.4.2. Adjusted aerobic respirometer data for acetone addition and control. Average and replicate data are presented for the four sediment samples.

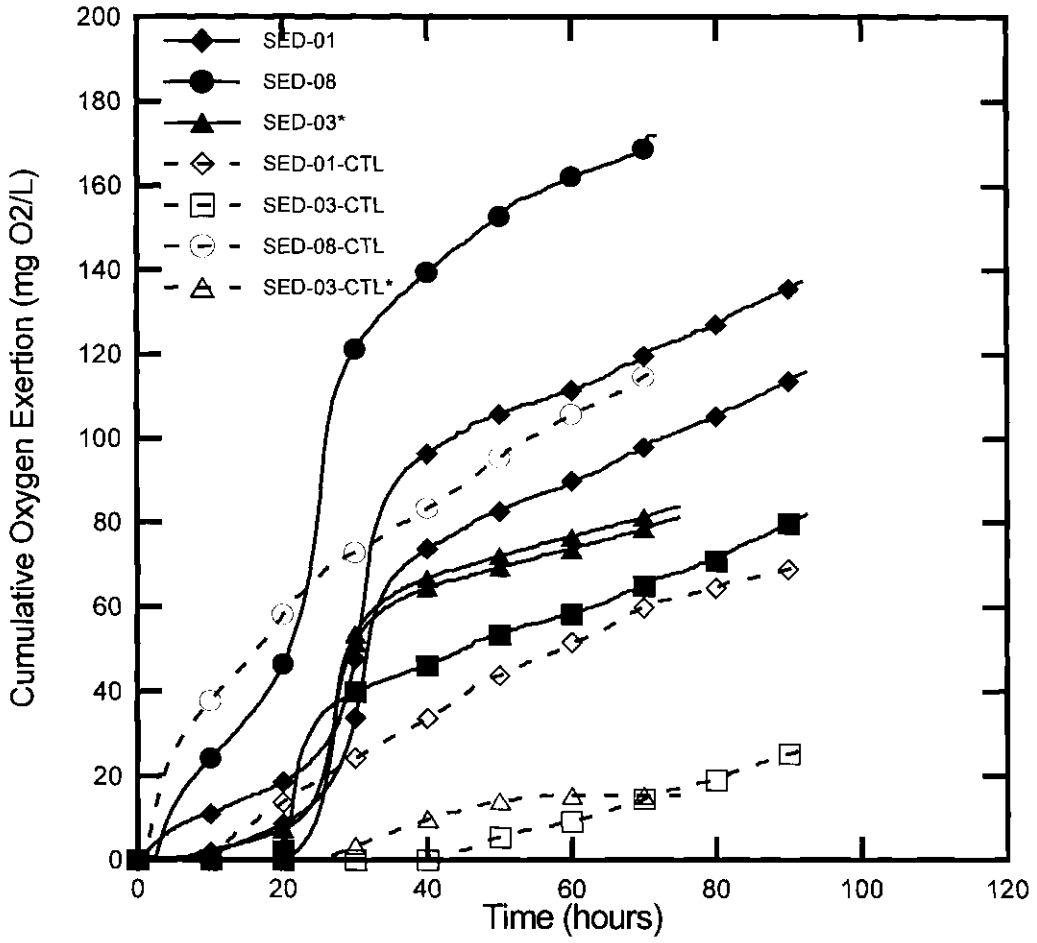


Figure 4.4.3. Aerobic respirometer data for phenol (100 mg O_2/L) addition and control at sediment concentrations of: SED-01, 44.2 g/L; SED-03, 72.2 g/L; SED-08, 47.1 g/L; SED-03*, 48.7 g/L (SED-03* denotes samples of NA-RAFB-0996-SED-03).

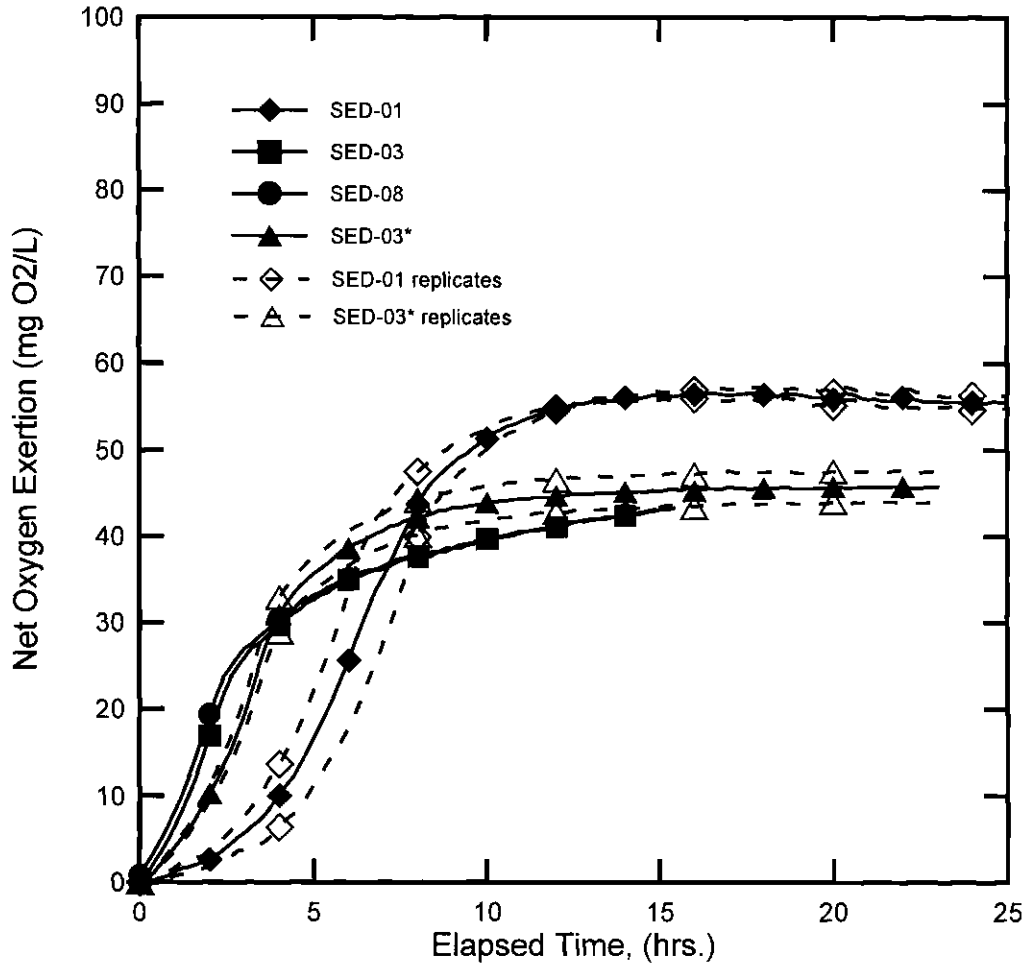


Figure 4.4.4. Adjusted aerobic respirometer data for phenol addition and control. Average and replicate data are presented for the SED-01 and SED-03* sediment samples; single-respirometric data are presented for SED-03 and SED-08.

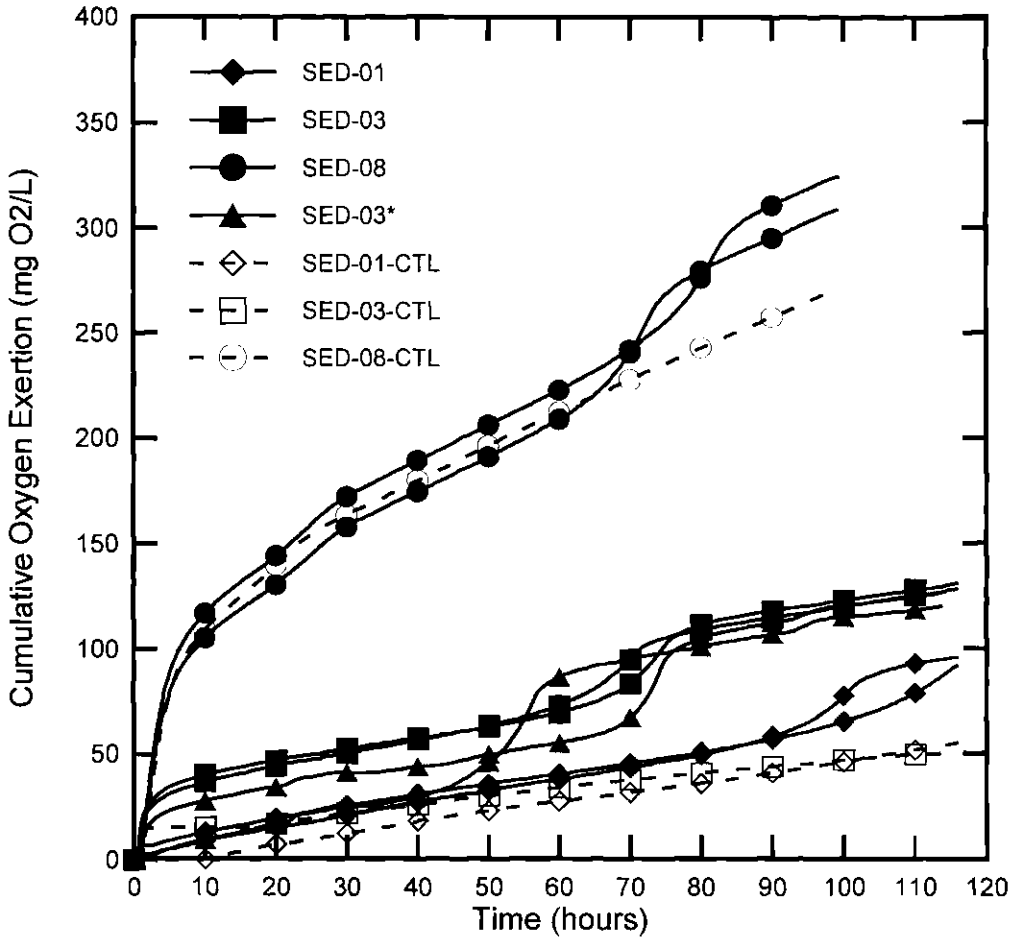


Figure 4.4.5. Aerobic respirometer data for benzene (100 mg O_2/L) addition and control at sediment concentrations of: SED-01, 44.1 g/L; SED-03, 70.9 g/L; SED-08, 80.1 g/L; SED-03*, 48.8 g/L (SED-03* denotes samples of NA-RAFB-0996-SED-03). There was no control for the SED-03* sample.

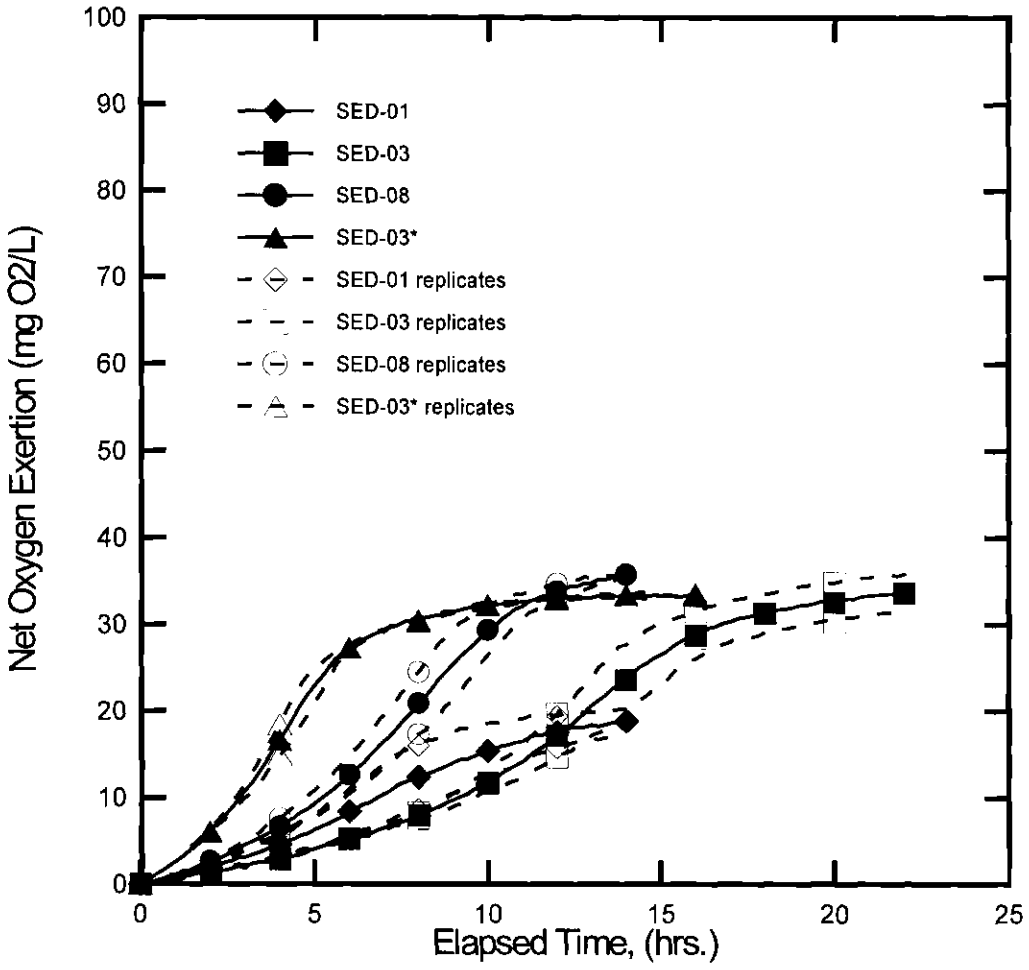


Figure 4.4.6. Adjusted aerobic respirometer data for benzene addition and control.

4.4.4. Chlorobenzene

SED-01, SED-03, SED-08, and SED-03* show oxygen exertion when chlorobenzene was added as substrate (Figures 4.4.7 and 4.4.8). In SED-01 sediments, chlorobenzene was utilized at faster rates than any other contaminant, and SED-03* shows chlorobenzene as the second fastest utilization rate, as presented in Tables 4.4.1 and 4.4.2.

4.4.5. 1,4-Dichlorobenzene

1,4-Dichlorobenzene was utilized by SED-01, SED-03, SED-08, and SED-03* as a growth substrate (Figures 4.4.9 and 4.4.10). Rates of 1,4-dichlorobenzene utilization indicate it was the slowest substrate utilized by SED-01 and SED-08, and the rates in SED-03 and SED-03* are similar to low benzene utilization rates. Although the rates are less than other contaminants, utilization by the indigenous microorganisms does occur and at rates of major significance in the context of utilization by wetland sediments.

4.4.6. Trichloroethylene

SED-01, SED-03, SED-08, and SED-03* show no oxygen exertion above background oxygen exertion when TCE was added as a substrate (see Figure 4.4.11). In some cases, the oxygen exertion in TCE experimental reactors was less than the control, suggesting some inhibitory effects on the indigenous aerobic microorganisms.

4.4.7. Kinetic Summary

The experimental data in Figures 4.4.2, 4.4.4, 4.4.6, 4.4.8 and 4.4.10 were examined to establish a first-order kinetic rate constant for contaminant oxidation. A non-linear regression technique (SigmaPlot graphics) was used with the general equation of $(dC)/(dt) = -k_a (C_t)$ with C_t = contaminant concentration at various times, t, and k_a = first-order rate constant for each contaminant under aerobic conditions. A standard error at the 95% confidence interval was determined with the technique and expressed in units of the rate constant. The rate constant was divided by slurry concentration, M, to normalize the kinetic rate to sediment concentration. The results of the first-order kinetic analysis are presented in Tables 4.4.1 to 4.4.4. SED-01 showed the fastest oxygen exertion rates, k_a, for chlorobenzene and the lowest k_a value for 1,4-dichlorobenzene. Phenol, acetone, and benzene rates were comparable. SED-03 readily utilized phenol and lower rates were seen with benzene and 1,4-dichlorobenzene. Phenol was also readily degraded by indigenous microorganisms in SED-08. 1,4-Dichlorobenzene was utilized at the slowest rate in SED-08 sediments. SED-03* samples showed the fastest rates when phenol was added as substrate and the next fastest rates with chlorobenzene as a substrate.

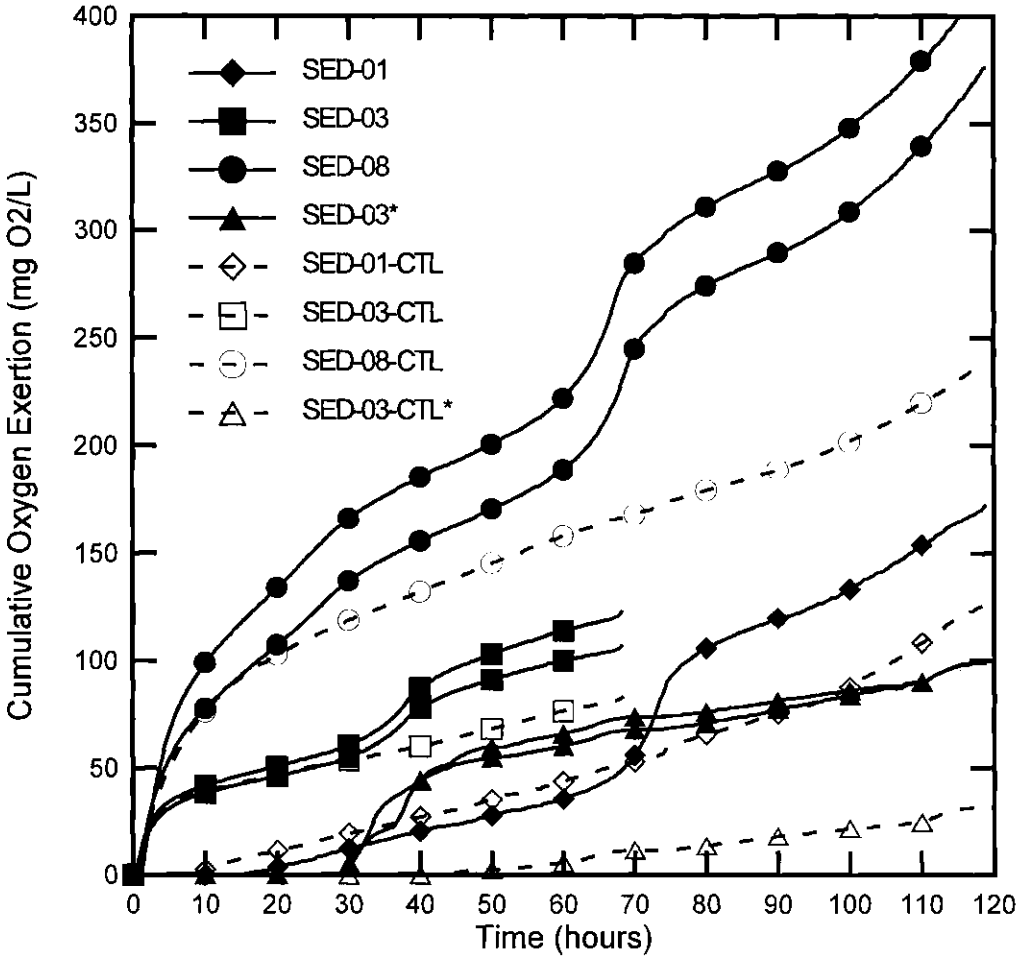


Figure 4.4.7. Aerobic respirometer data for chlorobenzene (100 mg O_2/L) addition and control at sediment concentrations of: SED-01, 44.1 g/L; SED-03, 75.0 g/L; SED-08, 77.3 g/L; SED-03*, 48.4 g/L (SED-03* denotes samples of NA-RAFB-0996-SED-03).

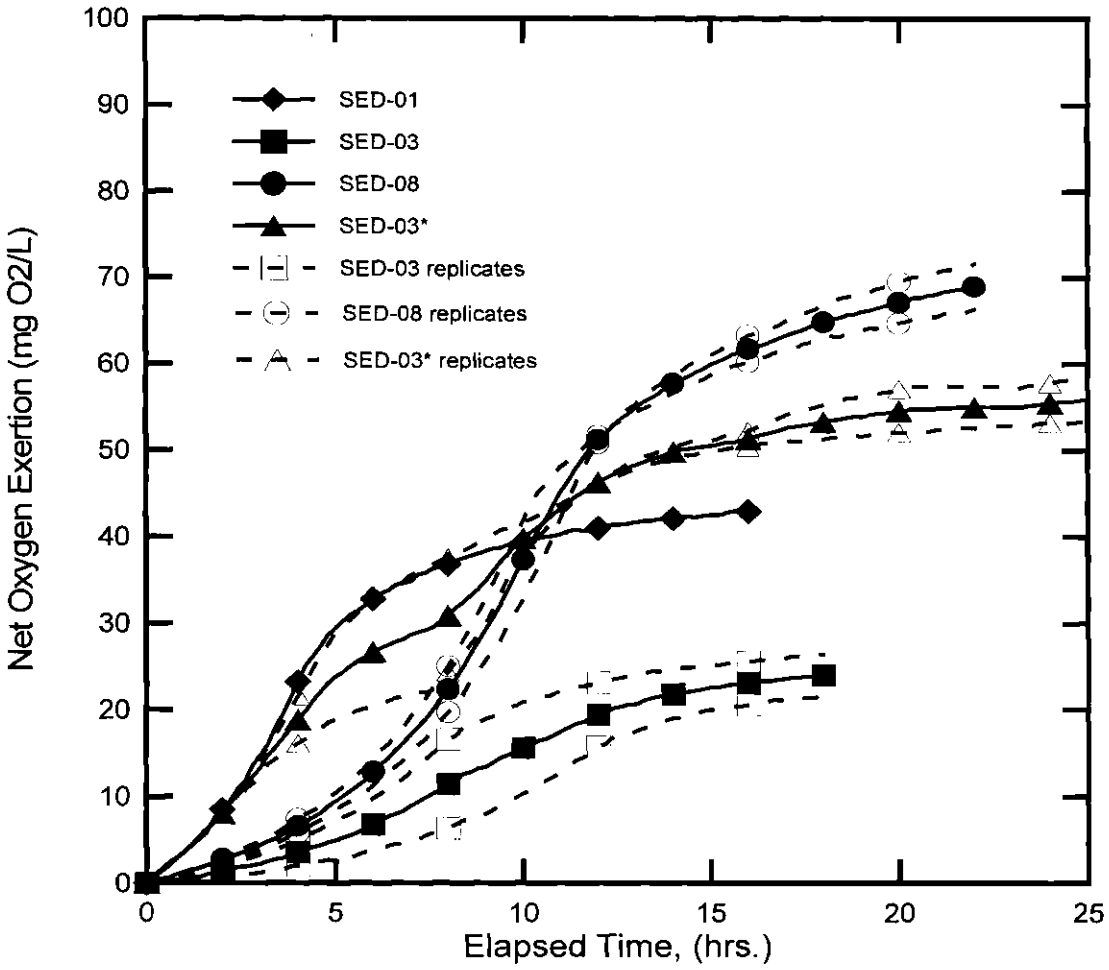


Figure 4.4.8. Adjusted aerobic respirometer for chlorobenzene addition and control.

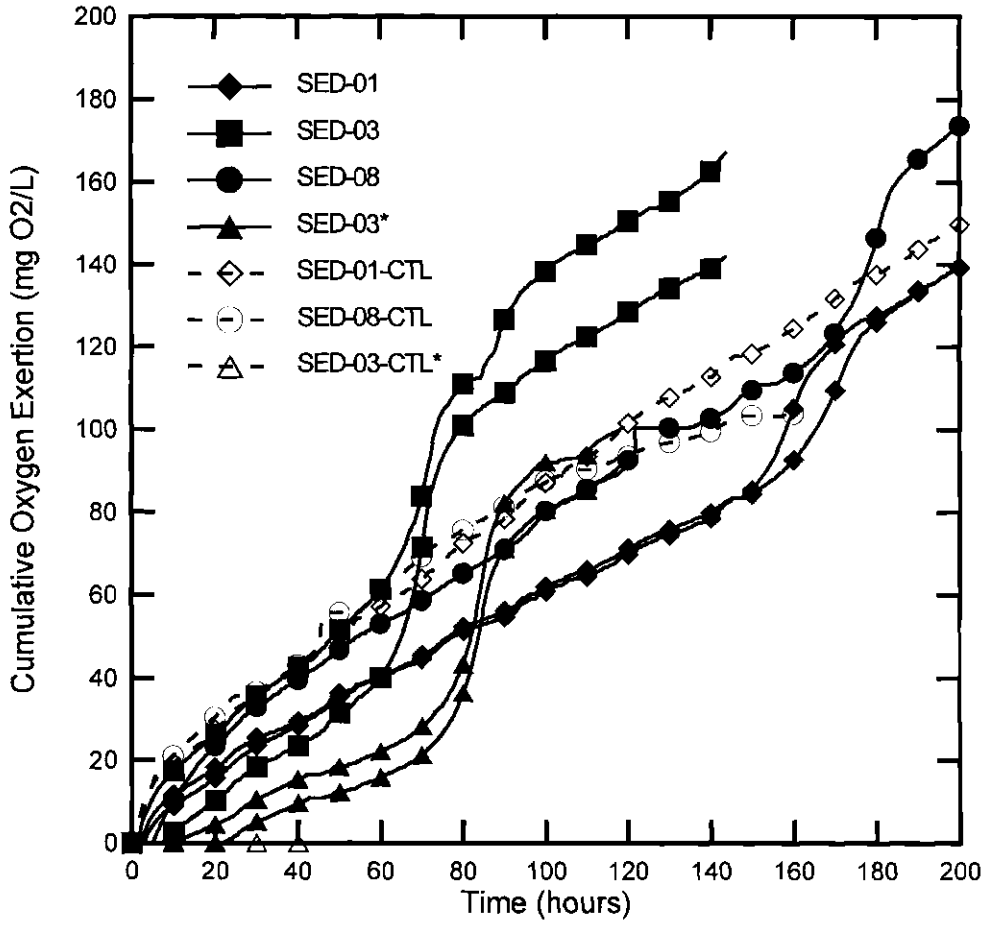


Figure 4.4.9. Aerobic respirometer data for 1,4-dichlorobenzene (100 mg O_2/L) addition and control for sediment concentrations of SED-01, 47.4 g/L; SED-03, 92.0 g/L; SED-08, 47.1 g/L; SED-03*, 48.8 g/L (SED-03* denotes samples of NA-RAFB-0996-SED-03).

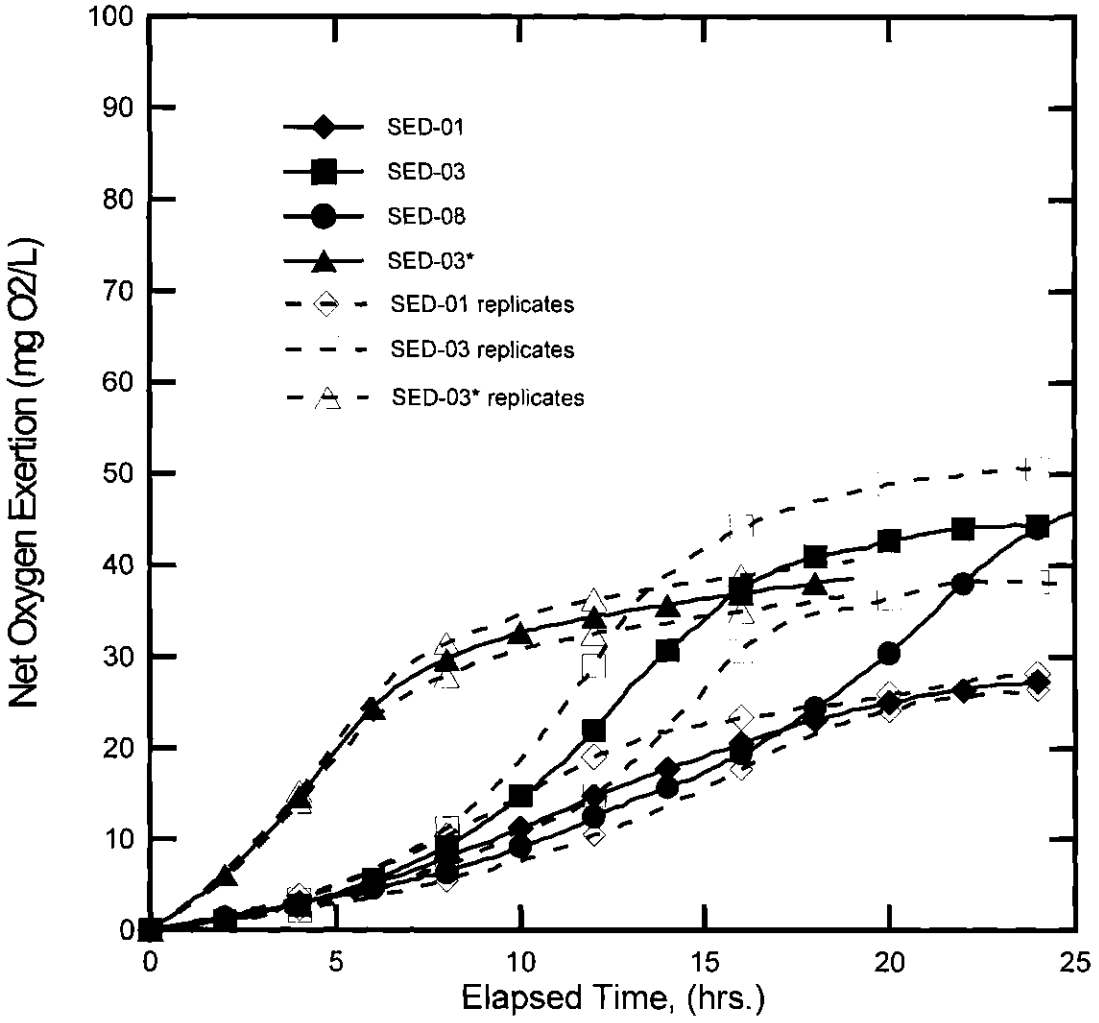


Figure 4.4.10. Adjusted aerobic respirometer data for 1,4-dichlorobenzene addition and control.

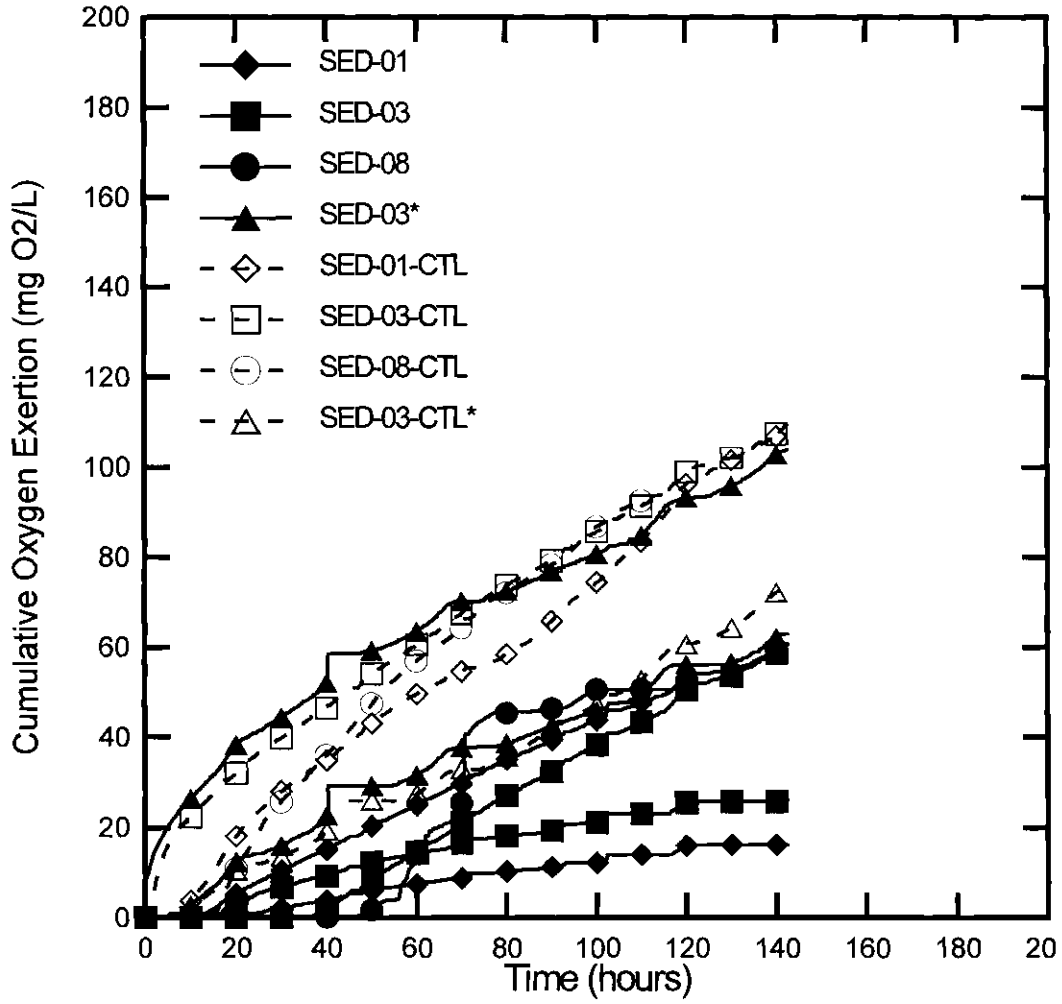


Figure 4.4.11. Aerobic respirometer data for trichloroethylene (100 mg O_2/L) addition and control for sediment concentrations of: SED-01, 30.0 g/L; SED-03, 48.7 g/L; SED-08, 29.4 g/L; SED-03*, 48.3 g/L (SED-03* denotes samples of NA-RAFB-0996-SED-03).

First Order Kinetic Data	First Order k_u		
NA-RAFB-0496 Samples:	Value (hr^{-1})	Std Error (hr^{-1})	CV (%)
SED-01:			
Acetone A	0.1607	0.00724	4.505
B	0.1370	0.00464	3.384
Benzene A	0.1080	0.00533	4.934
B	0.1574	0.00737	4.679
Chlorobenzene (CB) B	0.2114	0.00575	2.719
1,4-Dichlorobenzene (DCB) A	0.0623	0.00282	4.525
B	0.0861	0.00275	3.188
Phenol A	0.1385	0.00710	5.128
B	0.1761	0.00806	4.575
SED-03:			
Acetone A	0.1181	0.00275	2.329
B	0.1125	0.00219	1.943
Benzene A	0.0764	0.00389	5.095
B	0.0697	0.00339	4.862
Chlorobenzene (CB) A	0.0857	0.00428	4.992
B	0.1229	0.00517	4.205
1,4-Dichlorobenzene (DCB) A	0.0504	0.00129	2.556
B	0.0783	0.00387	4.947
Phenol B	0.2660	0.00396	1.390
SED-08:			
Acetone A	0.1181	0.00275	2.329
B	0.1125	0.00219	1.943
Benzene A	0.1035	0.00625	6.037
B	0.1332	0.00753	5.648
Chlorobenzene (CB) A	0.0965	0.00418	4.328
B	0.0837	0.00405	4.835
1,4-Dichlorobenzene (DCB) A	0.0119	0.00191	1.595
Phenol B	0.2877	0.00396	1.528

Table 4.4.1. Kinetic Summary for Aerobic Biodegradation of Specific Organic Contaminants for NA-RAFB-0496

First Order Kinetic Data	First Order k_1		
NA-RAFB-0996 Samples:	Value (hr^{-1})	Std Error (hr^{-1})	CV (%)
SED-03:			
Acetone A	0.0481	0.00204	4.226
B	0.0680	0.00291	4.272
Benzene A	0.0764	0.00383	5.019
B	0.0865	0.00380	4.395
Chlorobenzene (CB) A	0.1068	0.00266	2.493
B	0.1535	0.00227	1.478
1,4-Dichlorobenzene (DCB) A	0.0738	0.00249	3.375
B	0.0749	0.00223	2.973
Phenol A	0.2084	0.00893	4.287
B	0.2638	0.00809	3.067

Table 4.4.2. Kinetic Summary for Aerobic Biodegradation of Specific Organic Contaminants for NA-RAFB-0996

Normalized k_a values NA-RAFB-0496 Samples:	Slurry, M (g_{dry}/L)	k_a/M (L/(kg - hr))
SED-01:		
Acetone A	48.182	3.4
B	48.284	2.84
Benzene A	44.113	2.45
B	44.053	3.57
Chlorobenzene (CB) B	44.128	4.79
1,4-Dichlorobenzene (DCB) A	47.398	1.31
B	47.368	1.82
Phenol A	44.232	3.13
B	44.186	3.99
SED-03:		
Acetone A	74.629	1.58
B	74.413	1.51
Benzene A	70.905	1.08
B	71.137	0.98
Chlorobenzene (CB) A	75.033	1.14
B	74.905	1.64
1,4-Dichlorobenzene (DCB) A	91.982	0.55
B	92.010	0.85
Phenol B	72.191	3.68
SED-08:		
Acetone A	99.952	1.18
B	100.080	1.12
Benzene A	80.297	1.29
B	80.119	1.66
Chlorobenzene (CB) A	77.346	1.25
B	77.354	1.08
1,4-Dichlorobenzene (DCB) A	47.142	0.25
Phenol B	29.399	9.79

Table 4.4.3. Kinetic Summary of Aerobic Biodegradation of Specific Organic Contaminants for NA-RAFB-0496 Normalized to Slurry Concentration, M.

Normalized k_a values NA-RAFB-0996 Samples:	Slurry, M (g_{dry}/L_{media})	k_a/M (L/(kg - hr))
SED-03:		
Acetone A	66.873	0.72
B	66.925	1.02
Benzene A	48.841	1.56
B	48.815	1.77
Chlorobenzene (CB) A	48.352	2.21
B	48.368	3.17
1,4-Dichlorobenzene (DCB) A	48.778	1.51
B	48.649	1.54
Phenol A	48.661	4.28
B	48.766	5.41

Table 4.4.4. Kinetic Summary of Aerobic Biodegradation of Specific Organic Contaminants for NA-RAFB-0996 Normalized to Slurry Concentration, M.

Benzene and 1,4-dichlorobenzene had comparable rates and acetone was utilized at slower rates than other contaminants in SED-03*.

4.4.8. Additional Study of TCE in Aerobic Systems

The effects of TCE were examined with two additional phases of studies. The first phase investigated the effect of TCE addition, at various concentrations, on respiration of sediment microorganisms. A second phase was to evaluate the impact of TCE addition on the utilization of easily degraded compounds and a mixture of site contaminants by indigenous sediment microorganisms.

For the first phase, oxygen exertion through respiration processes of indigenous microorganisms was tested in 5% sediment slurries under aerobic conditions. TCE was added at the following μmole - levels: 11.1 (i.e., 1μL of TCE liquid to a 250mL slurry volume), 33 (3μL), 55.7 (5μL), 111.0 (10μL) and 334.4 (30μL) (Note: parenthetical volumes of 100% TCE were added to 250 mL reactor volumes, as summarized in Table 4.4.5). The maximum aqueous concentration levels, based on the calculation of full dissolution into the aqueous phase and on partitioning on soil and in water and air phases are included in Table 4.4.5. Two sediment controls without addition of TCE were tested as well. TCE data are typically presented as volumes (μL) added to the 250 mL aqueous volume.

TCE Added		TCE Conc. expressed as if dissolved completely in aqueous phase alone	Est. Aqueous-phase conc. of TCE*	TCE/sediment**
Vol. (μL)	Moles (μmole)	(mg/L)	(mg/L)	(μmole/g)
1	11.1	5.8	3.4	0.9
3	33.4	17.5	10.1	2.6
5	55.7	29.2	16.9	4.4
10	111.0	58.2	33.8	8.8
30	334.0	175.2	101.3	26.0

* equilibrium partitioning between aqueous and gaseous phased assumed

** full sorption to sediment-phase assumed

Table. 4.4.5. TCE additions to aerobic reactors in inhibition studies and estimated concentrations.

For the second phase, oxygen exertion through respiration processes of indigenous microorganisms was tested with 5% sediment slurry under aerobic conditions. Various levels of TCE were tested to evaluate the impact of TCE addition on the utilization of easily degraded compounds (5s) including benzoic acid, acetate, phthalate, ethanol, and p-cresol added at 100mg-COD/L levels (20 mg-COD/L each as previously described). For addition of five mixed specific site contaminants (5ss), a mixture of acetone, phenol, benzene, chlorobenzene and 1,4-dichlorobene was added at a level of 100 mg-COD/L level (20 mg-COD/L each). The TCE levels used for testing impact on 5s mixtures were 1 and 30μL (or 5.8 and 175.6 mg/L). The TCE levels used for testing 5ss mixtures were 1 and 10μL (or 5.8 and 58 mg/L). The tests for easily degraded compounds and site contaminants without addition of TCE were examined as well.

For the experimental results of the first phase, Figure 4.4.12 shows the original oxygen exertion over 120 hours for various levels of TCE addition (expressed in μL of TCE added) and sediment. The addition of TCE did not result in an increase in oxygen uptake. Hence, TCE was not biodegradable in the sediment, as indicated with previous sets of experiments. The trend furthermore is that increased TCE appeared to inhibit sediment microbial populations growing on sediment organic matter. Lag-adjusted data are included in Figure 4.4.13 and an analysis of the effects of TCE is possible using kinetic rates for these data. Zero-order kinetic rates for these TCE additions are summarized in Table 4.4.6. The kinetic rates for TCE levels of 1μL, 10μL and 30μL (5.8, 58.4 and 175.2 mg/L in Table 4.4.5) were slightly below that of the control, while

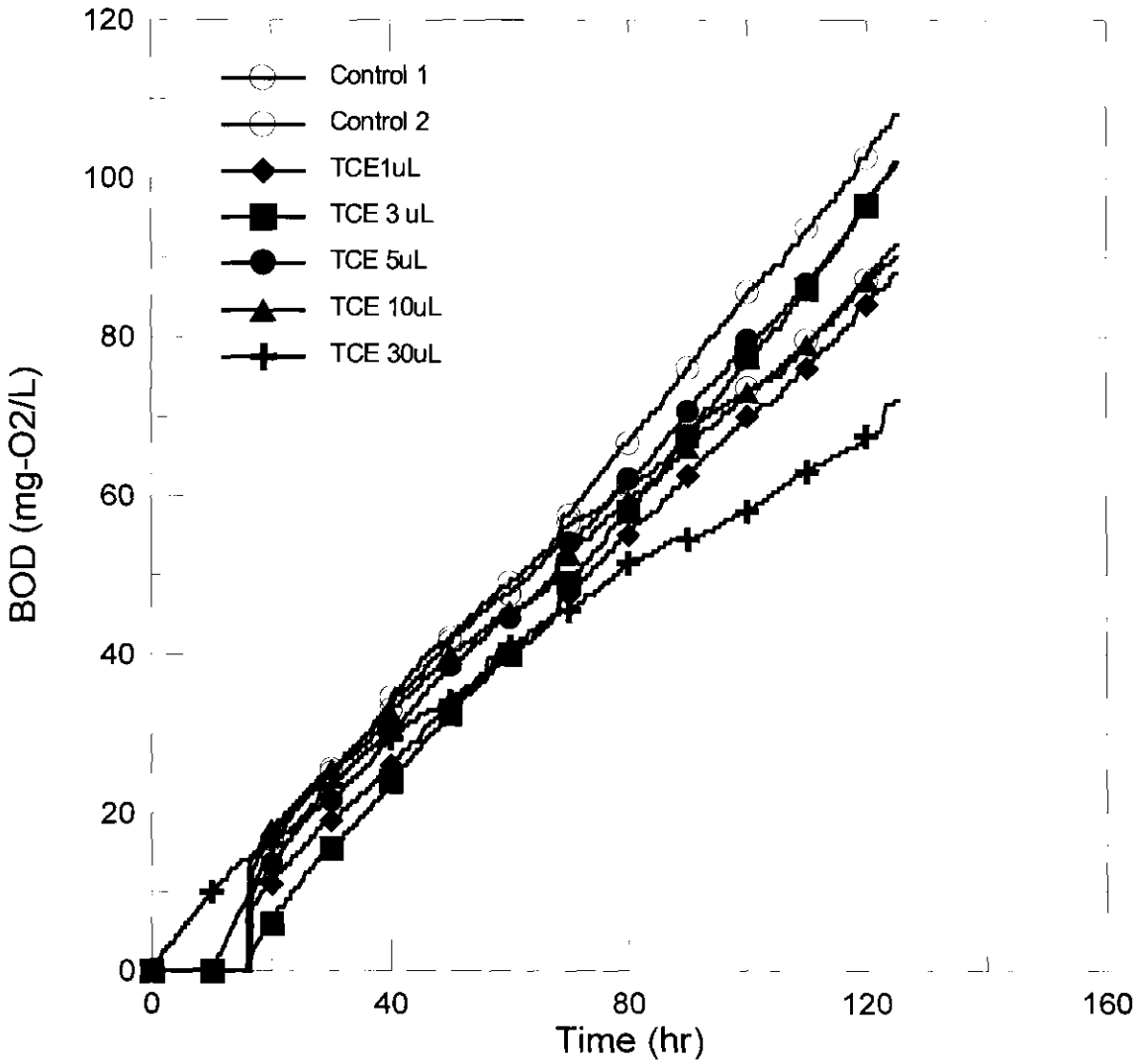


Figure 4.4.12. Oxygen utilization in sediment sample at approximate sediment concentrations of 50 g/L with TCE addition.

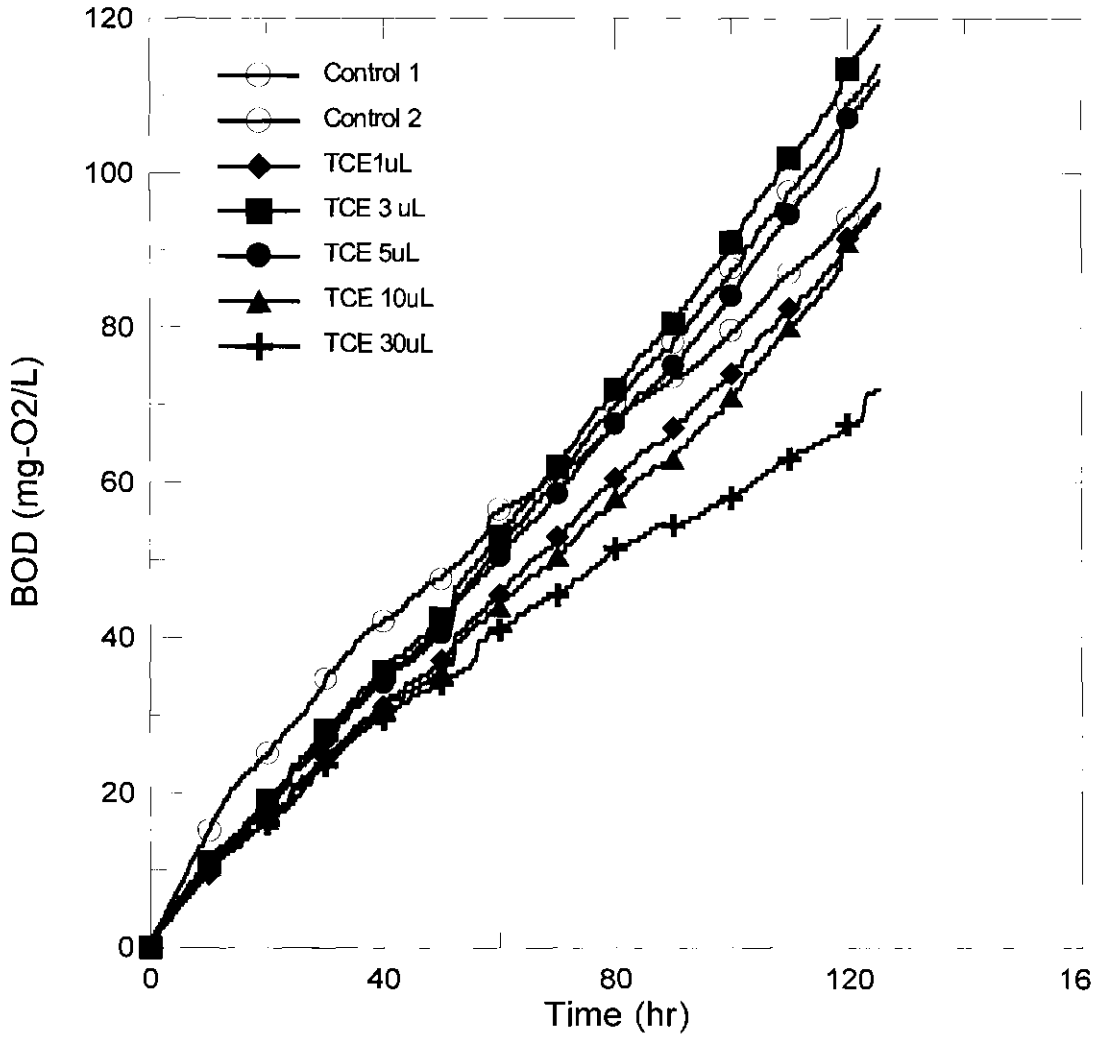


Figure 4.4.13. Data modified from original data by eliminating lag phase.

those for 3 and 5μL (17.5 and 29.2 mg/L) were slightly greater than the control. In the initial 60 hours of exposure to TCE at any level, oxygen uptake was inhibited (i.e., lower than the controls). TCE can potentially inhibit wetland microbial processes.

Sample	TCE conc.* mg/L	Slurry conc., M g/L	b_a, zero-order rate, mg/L·hr	R^2
Control	0	54.44	0.82	0.998
TCE1μL	5.8	55.49	0.752	0.999
TCE3μL	17.5	54.28	0.96	0.997
TCE5μL	29.2	55	0.900	0.996
TCE10μL	58.4	54.95	0.752	0.995
TCE30μL	175.2	54.16	0.518	0.993

* NOTE TCE conc. is presented as if all TCE was dissolved in aqueous phase to provide an equivalent conc. for comparison purposes.

Table 4.4.6. Kinetic summary of respirometer studies on TCE inhibition with addition of TCE only and modified

For the second phase of the study using other contaminant substrates with TCE, Figure 4.4.14 shows the original data of oxygen exertion utilizing easily degraded compounds (5s) and the mixture of five target contaminants (5ss) with and without addition of TCE. The original data were analyzed to eliminate the lag phase and the results were similar, with the exception of 5ss + TCE 10μL (58.4 mg/L)

For the sediments with easily degraded compounds and TCE additions of 0 μL,1 μL and 30μL, the consumption of oxygen in the initial 40-hour period was typical of previous studies with these substrates. Data in Figures 4.4.14 and 4.4.15 indicate TCE had no impact on the degradation of soluble substrates when added to the system. First-order kinetic rates for the three samples in Figure 4.4.16 were approximately 0.12hr^{-1} for the three samples.

For the addition of TCE with a mixture of the other four target contaminants (phenol, benzene, chlorobenzene and acetone at 20 mg/L per each), the response was complex, as presented in Figures 4.4.17 and 4.4.18. Maximum oxygen exertion for the samples with TCE ranged from 40 to 63 mg/L, compared to the control with a maximum of 56 mg/L. Clearly TCE affected the pattern of oxygen utilization but not the net degradation of the contaminants, based on oxygen consumption. Furthermore, employing the initial kinetic data (e.g., zero to 30 hours) the first-order rate constants from data in Figure 4.4.19 for the three samples were 0.083, 0.074 and 0.082 hr^{-1} for 0 μL,1 μL and 10 μL of TCE (Note: maximum oxygen-consumption for these rate constants are 60.7 mg-O_2/L, 60.7 mg-O_2/L and 22.5 mg-O_2/L, respectively, thereby indicating the differences in the prediction plots in Figure 4.4.19.).

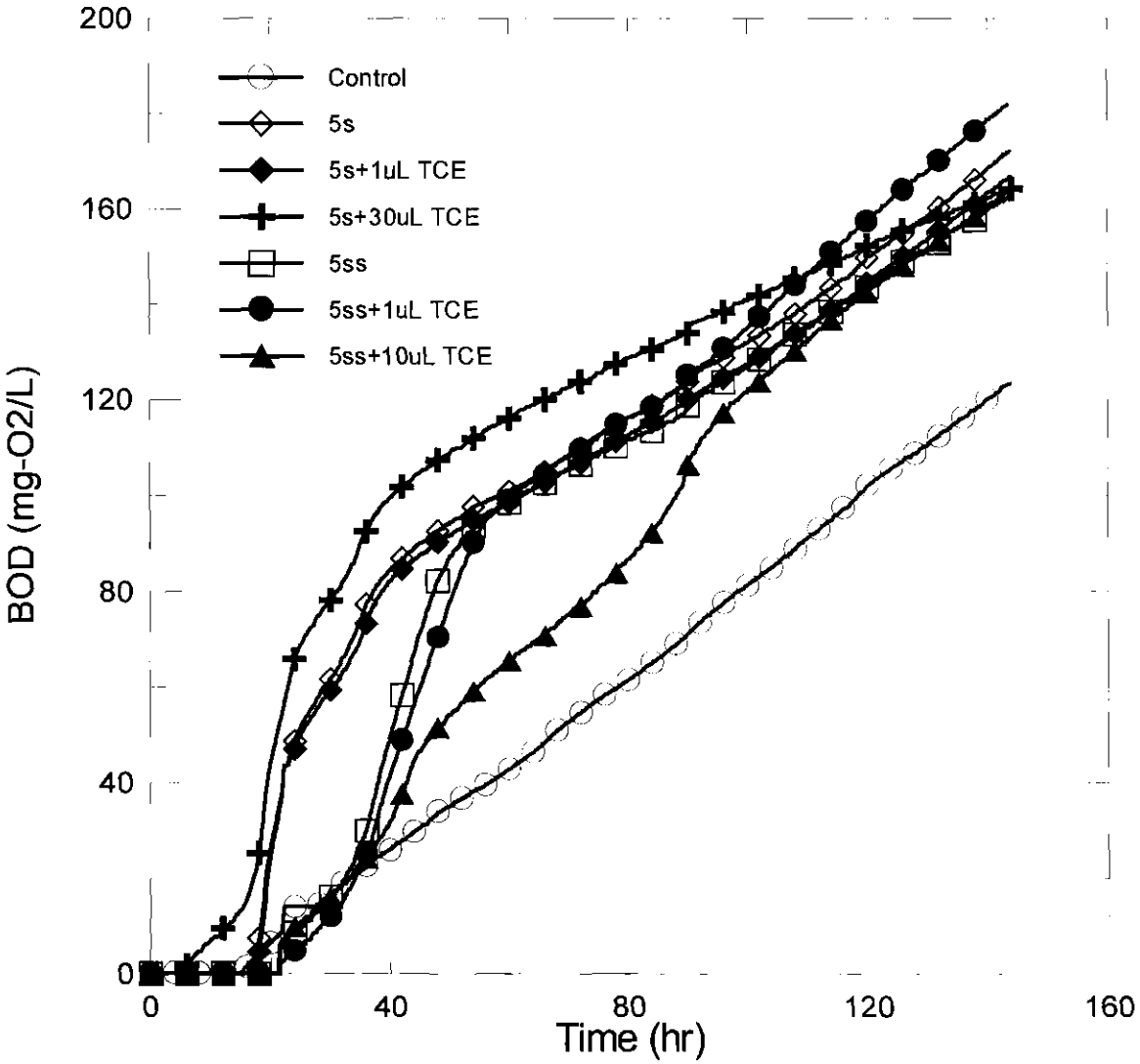


Figure 4.4.14. Respirometer results with addition of easily degraded compounds (5s) and mixed site contaminants (5ss) and TCE.

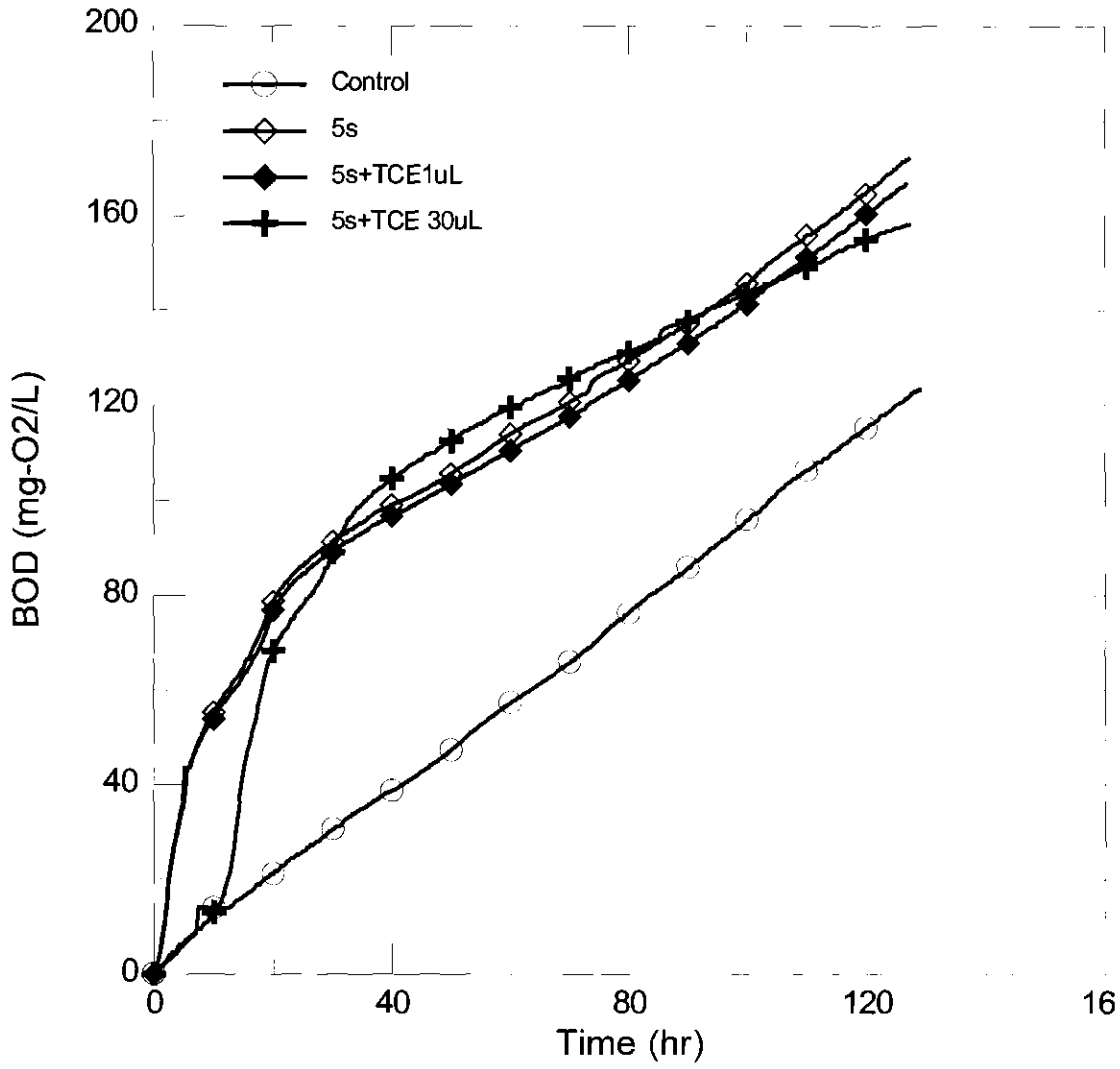


Figure 4.4.15. TCE respirometer results with concurrent addition of five easily degraded compounds; data have been modified to eliminate lag phase.

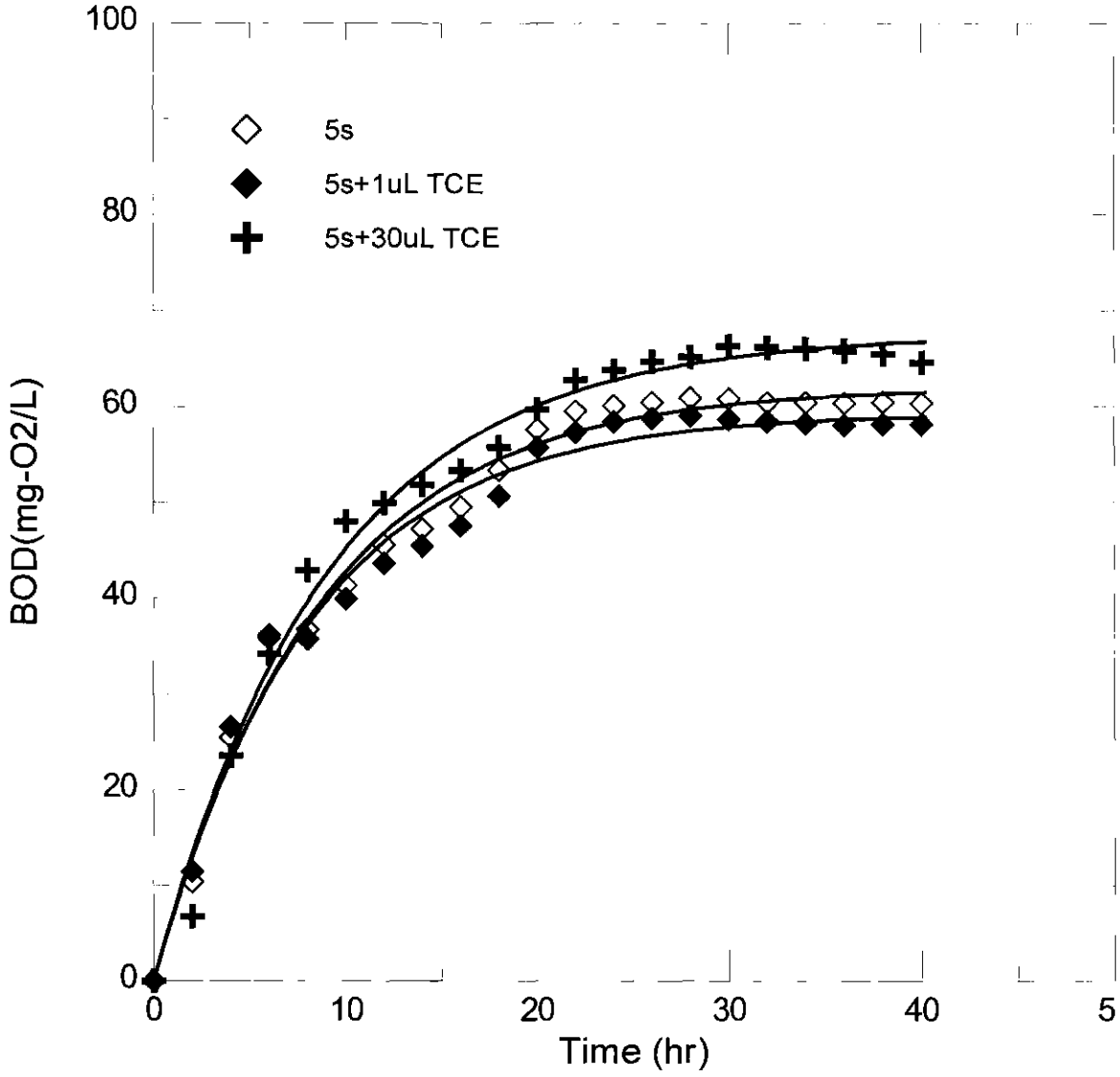


Figure 4.4.16. TCE respirometer results with concurrent addition of five easily degraded compounds and TCE. Data modified with sediment control and fit with first-order kinetic relationship.

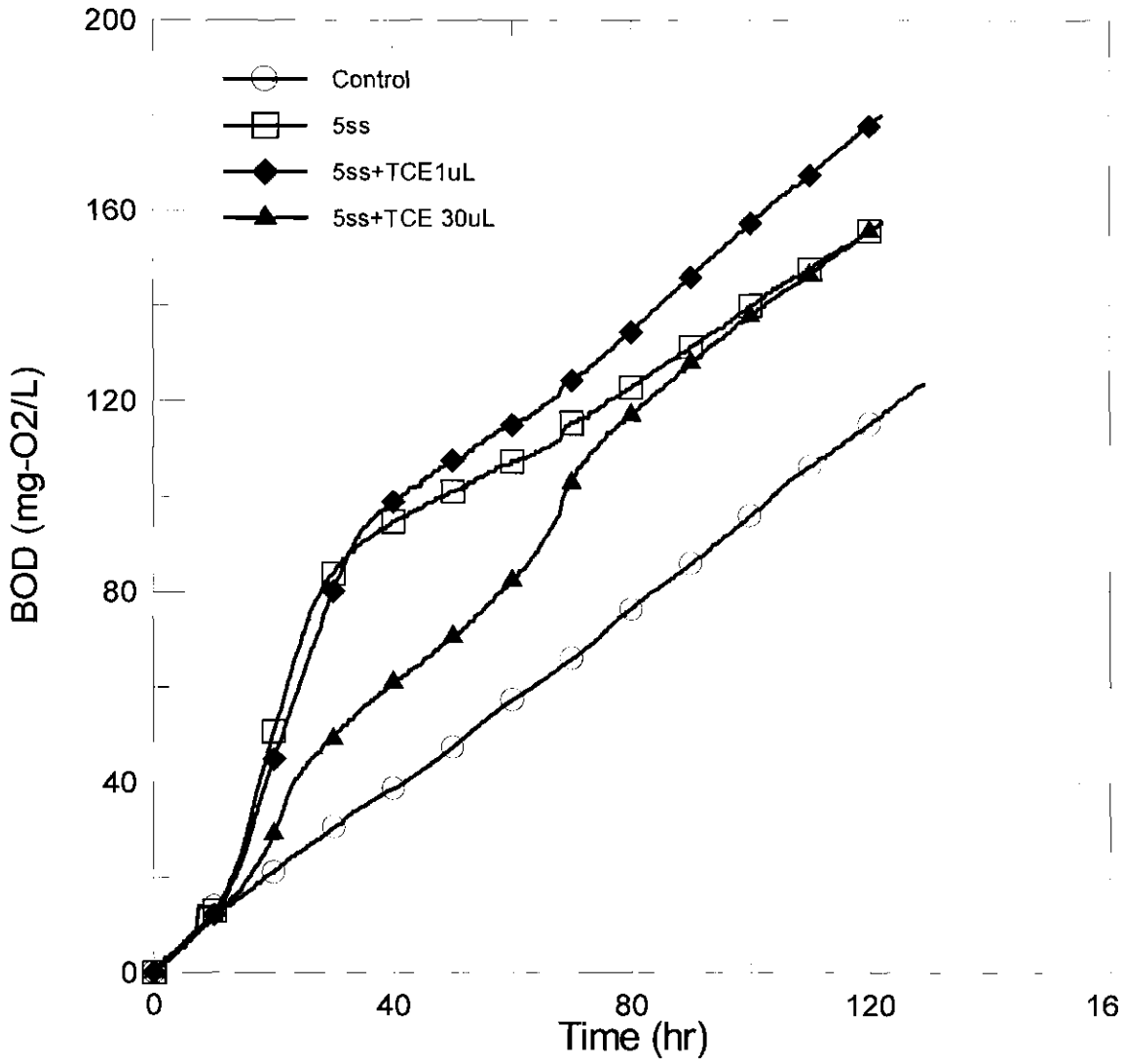


Figure 4.4.17. Respirometer results with additions of mixtures of site contaminants and TCE. Data modified by eliminating lag phase.

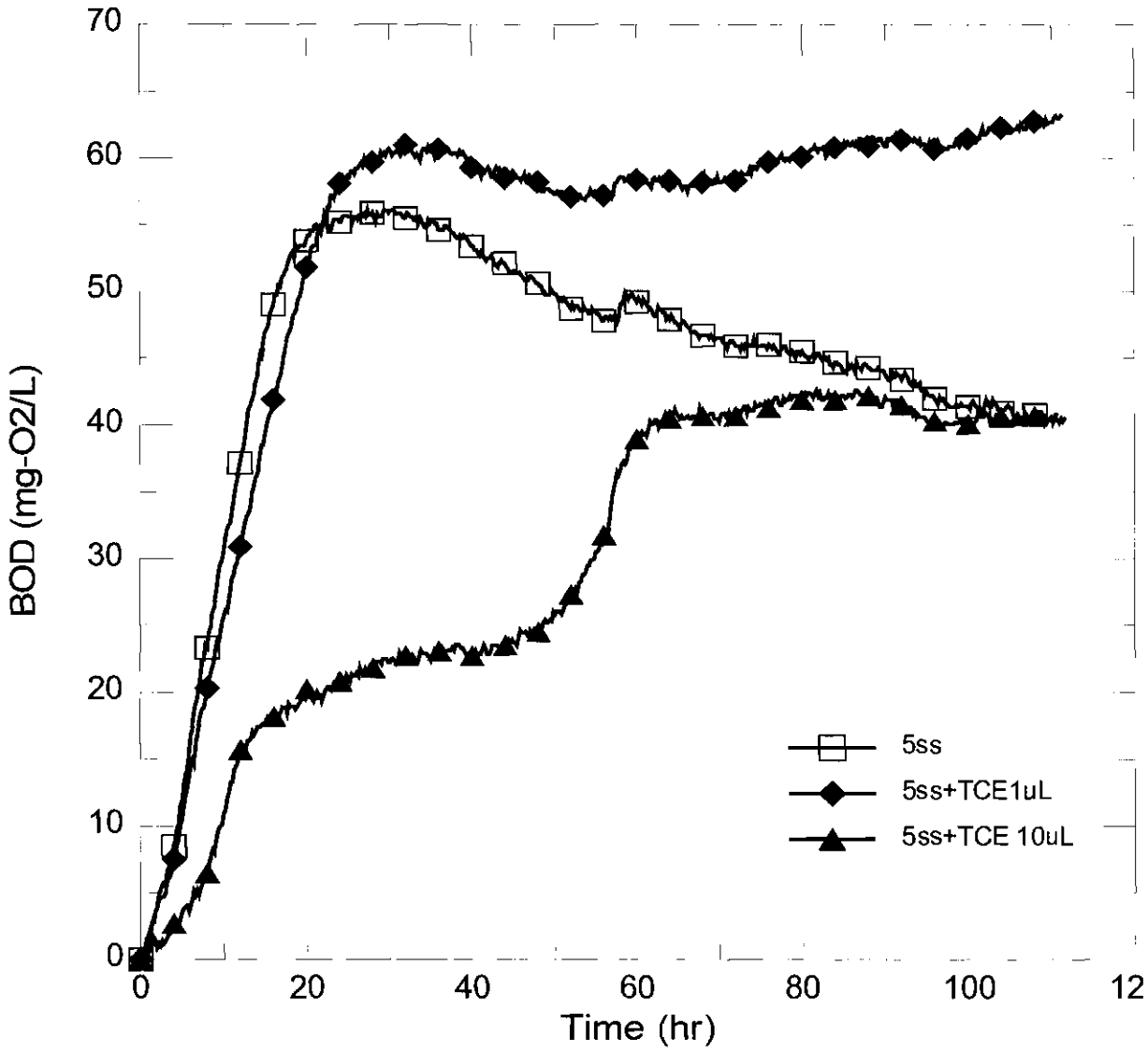


Figure 4.4.18. Respirometer results with additions of mixed site contaminants and TCE. Data modified with sediment control.

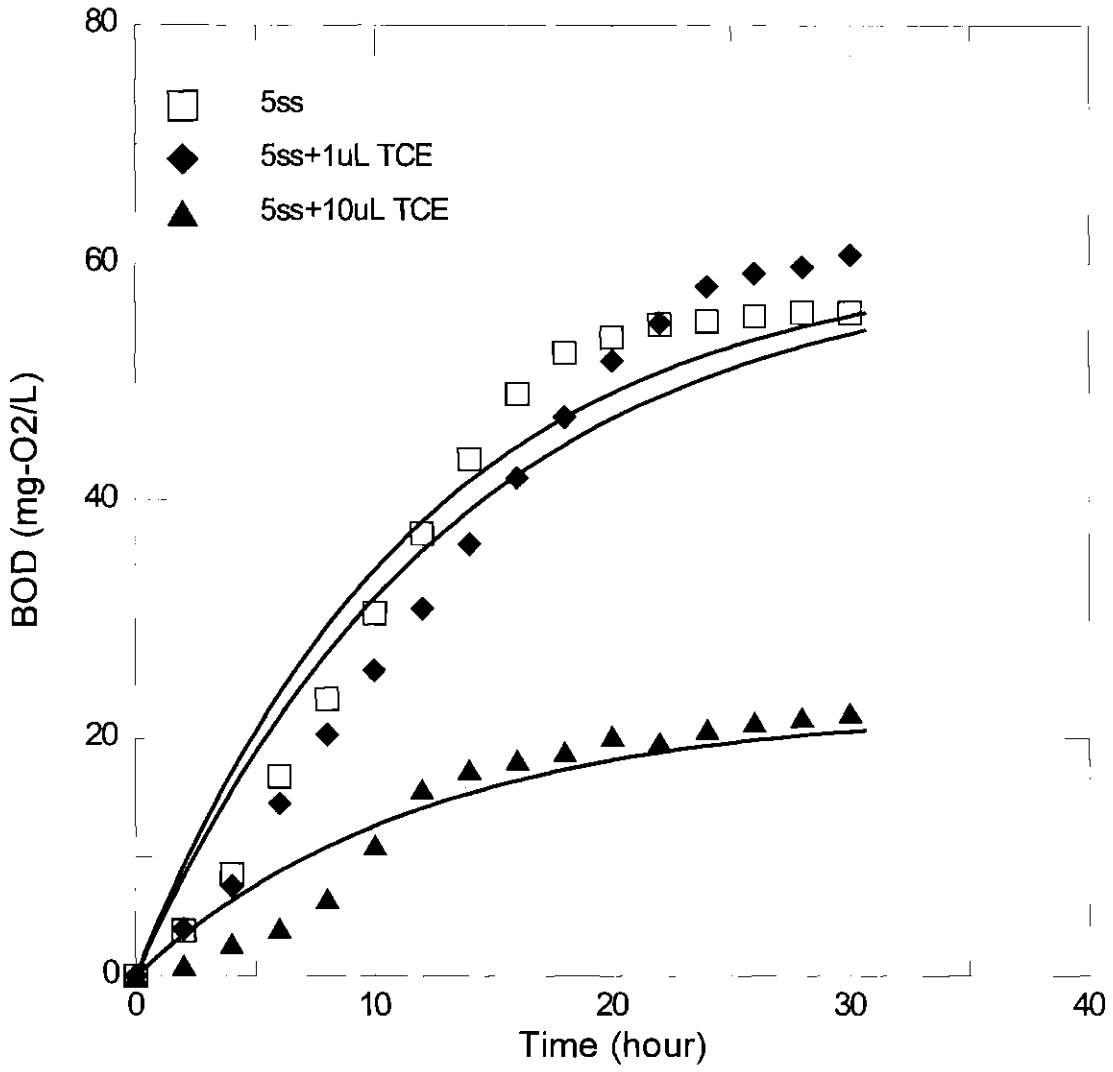


Figure 4.4.19. Respirometer results with addition of mixture of site contaminants and TCE. Data modified with sediment control and fitted into first order kinetic.

4.5. Anaerobic Biodegradation of Specific Organic Contaminants

Two phases of anaerobic study were included to illustrate the potential of using wetland sediments to biodegrade specific organic contaminants. One phase examined mineralization of specific organic contaminants. The mineralization processes can be predicted by the production of CO_2 from the utilization of specific organic contaminants by indigenous sediment microorganisms. [^{14}C]-labeled specific organic contaminants were used in this study. $^{14}CO_2$ produced from [^{14}C]-labeled specific organic contaminants was collected with a NaOH-trap system (Fig. 3.5.1). The overall concentration of [^{14}C]-labeled specific organic contaminant was negligible, compared to the total added target concentration (10 mg/L), thus the $^{14}CO_2$ production (and other ^{14}C-specific fractions) represented a portion of total CO_2 production (CO_2 + $^{14}CO_2$) in biological systems. The advantage of using [^{14}C]-labeled specific organic contaminant is that tracing the fate of [^{14}C]-labeled specific organic contaminant in anaerobic bioreactors can predict the behavior of specific organic contaminants in a system without interference of other biological processes, such as biodegradation of dissolved organic matter in natural wetland sediment and inorganic carbonate reactions, as well as avoidance of concerns for sorptive processes in establishing ultimate contaminant fate.

The concentration changes for a specific organic contaminant can not be predicted through these radiolabeled studies (i.e., injection of ^{14}C-labelled compounds in chromatographs is only permitted in ^{14}C-dedicated instruments, which were not included in those studies) in this study thus another phase of study using unlabeled specific organic contaminants was used to examine the degradation of specific contaminants versus time. For both phases of study, the initial added concentrations of specific contaminant was 10 mg/L. This concentration was for every contaminant added into bioreactors, therefore the wetland microorganisms were exposed to equivalent level of xenobiotic organic compounds, all of which were below any potentially toxic levels (see Table 1.2.2.). The experimental results on biodegradation of non-chlorinated aromatic hydrocarbons (acetone, phenol and benzene); chlorinated aromatic hydrocarbons (chlorobenzene and 1,4-dichlorobenzene); and chlorinated aliphatic hydrocarbon (TCE) using anaerobic sediment slurry systems are presented below.

4.5.1. Mineralization Studies of Radiolabeled Organic Contaminants

Mineralization studies using [^{14}C]-labeled specific organic contaminants were conducted with the total initial concentration of specific contaminants in each bioreactor being 10 mg/L. The concentration contributed from [^{14}C]-labeled material was negligible, but this [^{14}C] addition acted as a tracer to study the fate of specific organic contaminants in each active bioreactor. The fractions of [^{14}C] activity in each phase (i.e., supernatant liquid, sediment, headspace, stopper (sorbed) and CO_2) to initial addition of [^{14}C] activity also represented the fractions of unlabeled organic contaminant in each phase with respect to initial addition of 10 mg/L of a specific organic contaminant.

[^{14}C] activities in liquid, solid and gaseous samples of the anaerobic slurry system were determined on a Beckman LS6500 scintillation counter. The methods to analyze the fractions of [^{14}C] activity in the volatile (gaseous phase), nonvolatile (soluble phase and/or sorbed phase on soil particles) phases to initial addition of [^{14}C] activity and fraction of $^{14}CO_2$ production to initial addition of [^{14}C] activity were described in section 3.5.1. Note that the initial additions of 10 mg/L of unlabeled contaminants were to observe the response of active microorganisms under the same exposure condition (10 mg/L for each specific organic contaminant). Even though they were spiked into each individual bioreactor with a consistent manner, these concentrations were calculated based on the volume added manually each time and they were not obtained from instrumental analyses. ^{14}C-labeled studies were conducted with acetone, benzene chlorobenzene and dichlorobenzene. TCE and phenol were not examined due to unavailability of the labeled compounds and because of the volatility of the TCE relative to $CO_{2\,(g)}$, causing analytical complications.

[$^{14}CO_2$] Production and Fate of [^{14}C]-Acetone. Acetone is a compound with high solubility in water and it is therefore expected that acetone has minimum distribution in gaseous or sediment-solid phases. [^{14}C] activities displayed as fractions (%) of recovered [^{14}C] activities to initial addition of [^{14}C] activity should be low in the sediment and gaseous phases. This is indicated in Figure 4.5.1. Low [^{14}C] activity was recovered in head space, stopper and sediment phases and approximately 95% of [^{14}C] activity was found in the supernatant fluid which included dissolved CO_2, acetone and degradation products.

Data in Figure 4.5.2 include a separation of the soluble-acetone component and the CO_2 component in the supernatant-fluid phase (from Figure 4.5.1). It is clear that mineralization of the acetone occurred and that the net CO_2 was attributable to acetone and its decomposition products. The mineralization and complete decomposition of [^{14}C]-radiolabeled acetone was completed in 50 days and the recovered $^{14}CO_2$ occupied averaged 95% of overall [^{14}C] activity. This radiolabeled study confirmed that acetone can be biodegraded under anaerobic conditions within 50 days (in a 5% sediment slurry). Ten days of lag phase can be explained by the acclimation of bacteria to the optimized nutrient-rich sediment slurry system.

[$^{14}CO_2$] Production and Fate of [^{14}C]-Benzene. Benzene is a compound with solubility of 1,789 mg/L, log K_h equal to 0.74 and log K_{ow} equal to 2.13. K_h, Henry's constant, represented the partitioning of benzene in gaseous and water phases, and K_{ow} indicates partitioning of benzene in octanol and water phases. It is common to predict organic compound adsorption by soils using K_{ow}, with the higher values having higher tendency for that compound to be adsorbed by soils. Benzene, however, has high K_h and K_{ow}. Thus, significant distribution of benzene in sediment and gaseous phase was expected. Figure 4.5.3. represents the fractions of [^{14}C] activity to initial added [^{14}C]-labeled benzene in each phase. Fifty percent of [^{14}C] activity was recovered in supernatant fluid and 25% of [^{14}C] activity was in the sediment phase during the experimental period. [^{14}C] activity in stopper and headspace phases ranged from 0 to 5%.

The overall recovered [^{14}C] activity was 65-75% and was lower than expected. The loss of [^{14}C] activity can be contributed to the limitation of instrumentation or experimental procedures. The scintillation counting of stopper and sediment may not be precise because radiation from scintillation counter may be unable to penetrate through the stopper and sediment particles and then the radiation was blocked. The loss due to experimental procedures may also be caused by transferring samples. Nonetheless, the purpose was to establish the fate of benzene-carbon with respect to mineralization to CO_2.

$^{14}CO_2$ production was determined by purging a caustic-treated supernatant sample through a NaOH trap system. No mineralization ($^{14}CO_2$ production) of [^{14}C]-labeled benzene was observed. It is noted that recovered fraction of $^{14}CO_2$ was included in Figure 4.5.3., however, when [^{14}C] activity in supernatant fluid was caustic-treated and measured, $^{14}CO_2$ dissolved in supernatant fluid was then separated from supernatant fluid and collected through NaOH trap system. Therefore, $^{14}CO_2$ data were not counted into the overall recovery efficiency of [^{14}C] activity. In summary, benzene was not mineralized in the sediments.

[$^{14}CO_2$] Production and Fate of [^{14}C]-Chlorobenzene. Chlorobenzene has a much lower solubility (503 mg/L) than benzene, hence the chlorobenzene would not prefer to stay in water phase if other favorable phases are available. Its Log K_h and Log K_{ow} are 0.55 and 2.92, respectively. Consequently, it was observed from Figure 4.5.4. that the fraction of [^{14}C] activity in supernatant phase dropped to 35-40% and the fraction raised to 40% in sediment phase compared to the observations in study of [^{14}C]-labeled benzene. The fraction of [^{14}C] activity in stopper was significant (5-7%) compared with previous [^{14}C]-labeled acetone and [^{14}C]-labeled benzene studies. Furthermore, [^{14}C] activity in headspace was low. The low [^{14}C] activity in headspace was explained by the existence of sediment phase which was organic-rich (hydrophobic) and had high surface area to adsorb chlorobenzene and therefore overcome the chance of volatilization into headspace. These observations predicted that chlorobenzene was more hydrophobic, not willing to dissolve in water and less bioavailable to microorganisms. Again, $^{14}CO_2$ collected in NaOH trap was negligible (Fig. 4.5.4). No mineralization of chlorobenzene was observed in the sediments.

[$^{14}CO_2$] Production and Fate of [^{14}C]-1,4-Dichlorobenzene. Of the organic contaminants tested, 1,4-dichlorobenzene has the lowest solubility (59.88 mg/L), lowest Log K_h (0.35) and highest Log K_{ow} (3.38). These characteristics of 1,4-dichlorobenzene express the possible behavior in closed sediment systems. In particular, dissolution limits mass transfer of 1,4-dichlorobenzene and its availability to microbial degradation.

The fraction of [^{14}C] activity in the sediment phase was 40-50% and in supernatant phase was 30%. The fraction in stopper was around 5% and in headspace was almost negligible. No mineralization of 1,4 dichlorobenzene occurred (Figure 4.5.5).

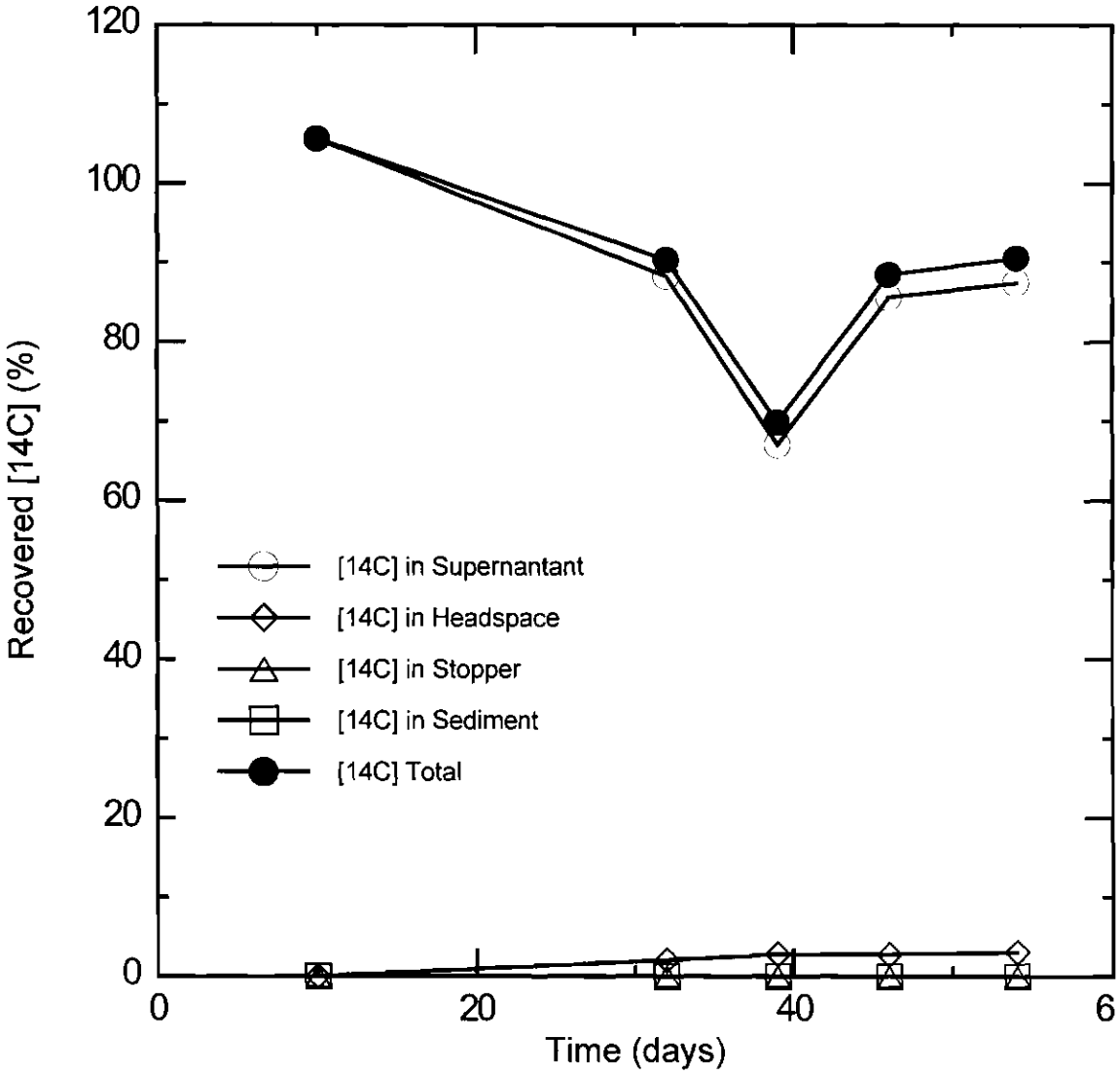


Figure 4.5.1. Distribution of ^{14}C-acetone using NA-RAFB-0996-SED-03 sediment under methanogenic conditions. Total acetone concentration was 10.8 mg/L and sediment concentration was 49g/L.

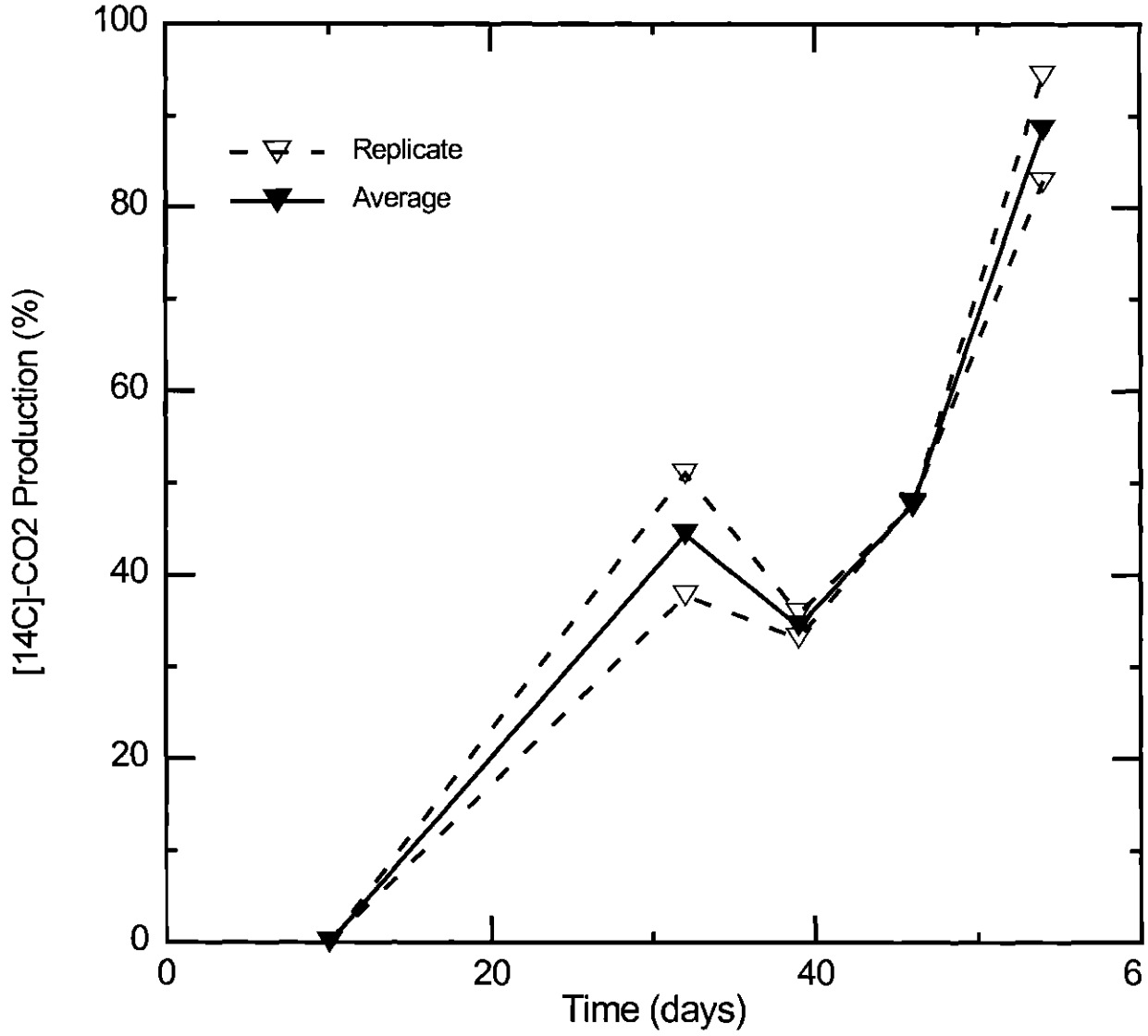


Figure 4.5.2. Mineralization of ^{14}C-acetone to CO_2 using NA-RAFB-0996-SED-03 sediment under methanogenic conditions.

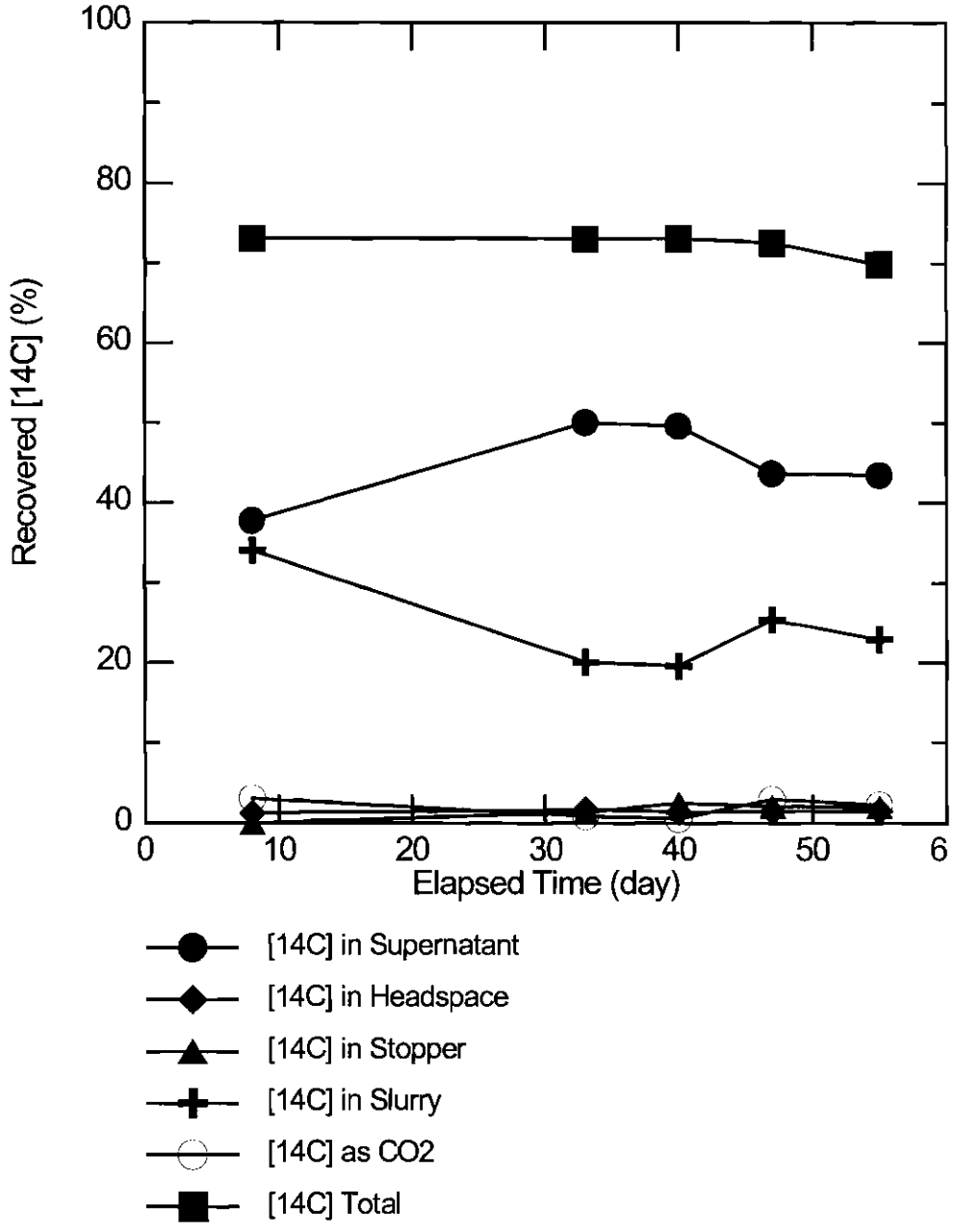


Figure 4.5.3. $^{14}CO_2$ production and distribution of ^{14}C-benzene using NA-RAFB-0996-SED-03 sediment under methanogenic conditions. Total initial benzene concentration was 10.3 mg/L and sediment concentration was 52 g/L.

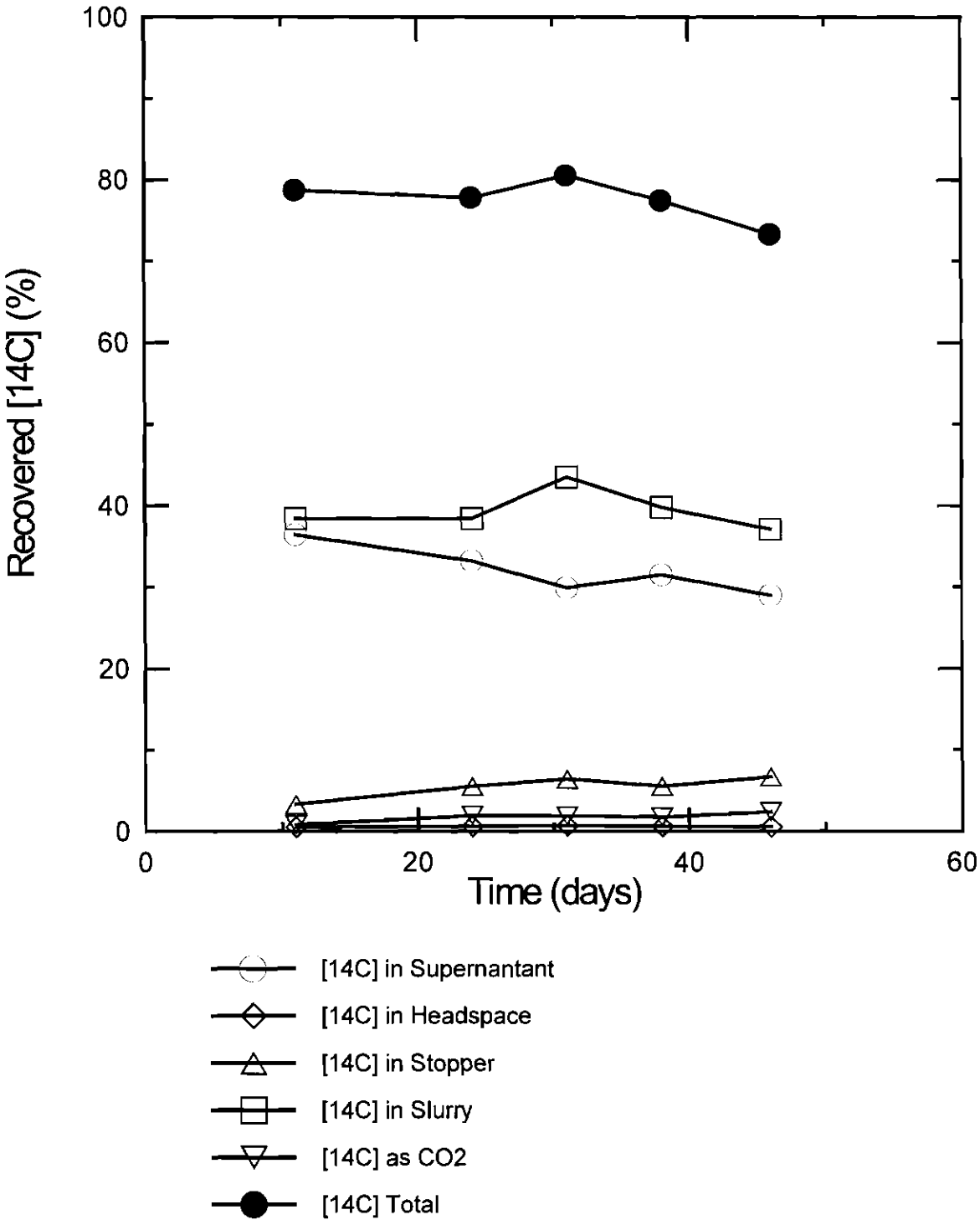


Figure 4.5.4. $^{14}CO_2$ production and distribution of ^{14}C-chlorobenzene using NA-RAFB-0996-SED-03 sediment under methanogenic conditions. Total initial chlorobenzene concentration was 10.8 mg/L and sediment concentration was 49 g/L.

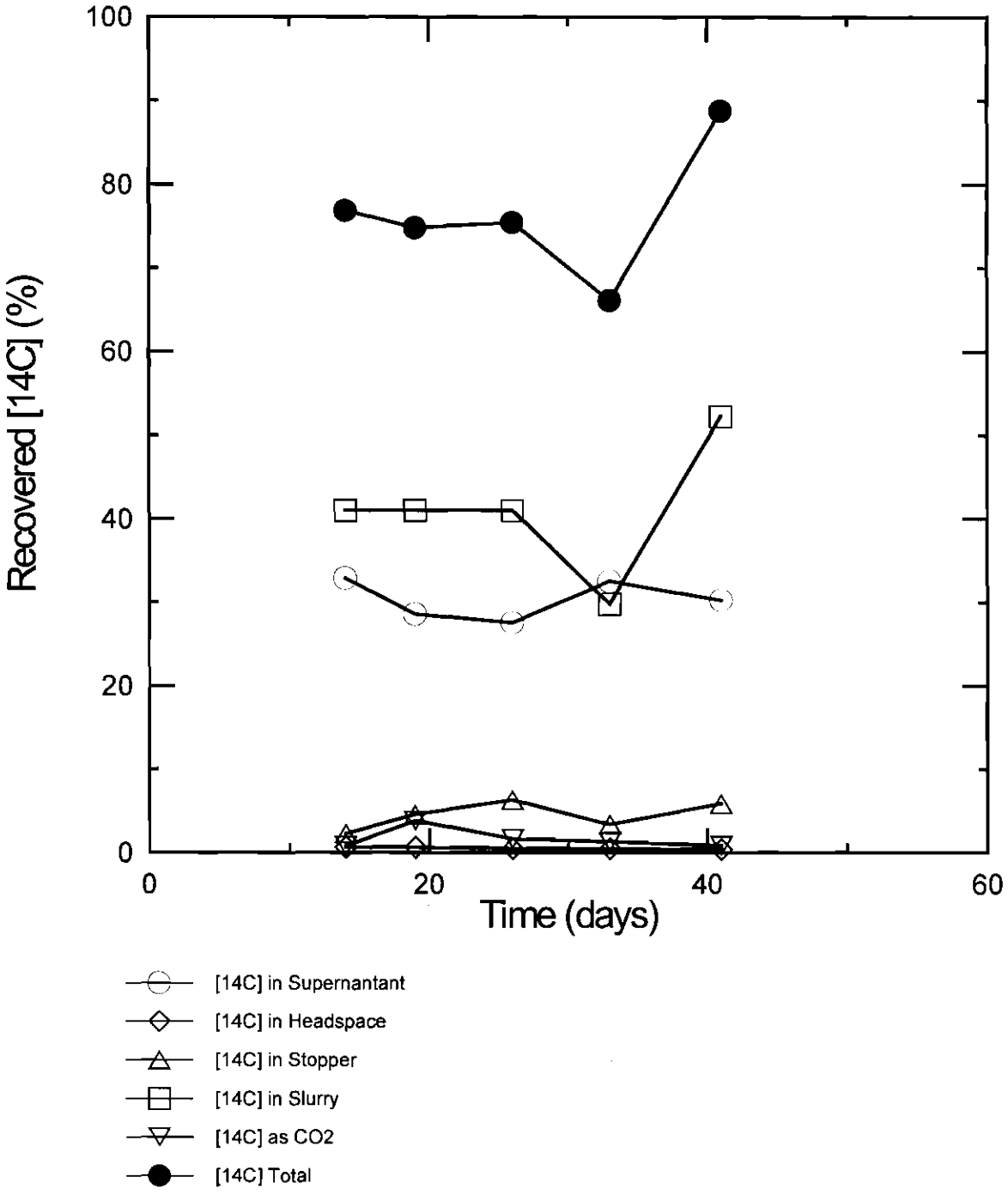


Figure 4.5.5. $^{14}CO_2$ production and distribution of ^{14}C-1,4-dichlorobenzene using NA-RAFB-0996-SED-03 sediment under methanogenic conditions. Total initial 1,4-dichlorobenzene was10.9 mg/L and sediment concentration was 49 g/L.

Kinetic Summary of [$^{14}CO_2$] Production. It was confirmed using studies of [^{14}C]-labeled specific organic contaminants that benzene, chlorobenzene and 1,4-dichlorobenzene cannot be mineralized by indigenous microorganisms under anaerobic conditions. Acetone can be effectively mineralized.

4.5.2. Anaerobic Studies of Unlabeled Organic Contaminants

The potential biodegradation of acetone, benzene, chlorobenzene, 1,4-dichlorobenzene, phenol and TCE using wetland sediments under methanogenic conditions was examined with a serum-bottle system. In these studies, specific site contaminants were measured with gas chromatography. Based on the study of radiolabeled experiments, it was confirmed that chemical distributions in headspace and stopper are negligible because of the organic-rich sediment which has capability to adsorb significant amount of chemicals and thereby reduce the potential of chemicals to be volatilized. Based on previous data on contaminant partitioning, contaminants were monitored only in the aqueous and solid phases.

The mass of chemical in aqueous and sediment phases extracted with organic solvent are presented as a percentage (%) of extractable chemical from each phase relative to original chemical added. Note that the mass recovered from sediment phase is normalized to dried sediment. Furthermore, chemicals extracted from abiotic sediment controls are presented together with biotic sediments as a measure of total chemical recovered.

Methane production of indigenous microorganisms exposed to each spiked specific contaminant is accessed and therefore can indirectly indicate the effect of spiked specific contaminant on microbial activity. Therefore, two parallel assessments are included within this experimental procedure. First, the effect of contaminant on methane production is assessed. If methane production is stimulated, this would indicate enhanced metabolism. No effect on methane production could be attributed to no bioassimilation of the contaminant or insufficient net production of methane (and CO_2) relative to that produced from natural organic matter in the sediments. A negative impact on methane production would indicate an inhibitory response on the current microbial population. Secondly, assimilation of the compound and its resulting removal from the aqueous- and sediment-phases would indicate an initial transformation of the compound towards a terminal endproduct (e.g., CO_2, CH_4 or cellular biomass). It however is not conclusive of biodegradation since intermediate endproducts may accumulate and/or become sorbed within the sediment matrix. Detailed delineation of endproducts was not, however, within the scope of this research. A favorable response with respect to biodegradation would be a clear positive impact on methane (or CO_2) production and contaminant removal from the aqueous and sediment phases.

Acetone. Evidence of biodegradation of acetone is indicated by the complete mineralization and production of $^{14}CO_2$ during radiolabeled experiments (Figure 4.5.2). Methane production by utilizing acetone is presented in Figure 4.5.6. Average methane

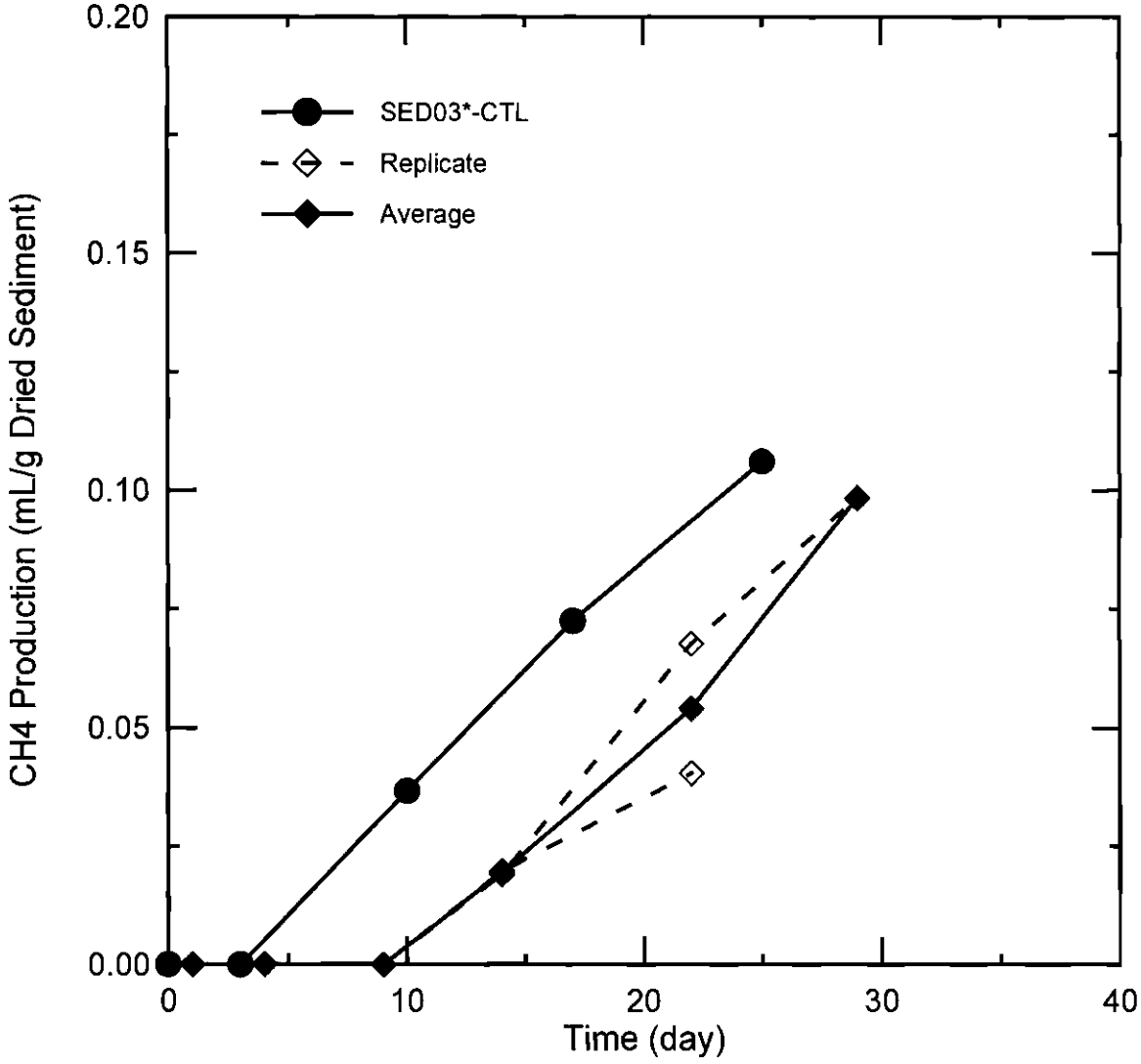


Figure 4.5.6. CH_4 production from acetone using NA-RAFB-0996-SED-03 sediment under methanogenic conditions. Total initial acetone concentration was 20 mg/L and sediment concentration was 52 g/L.

productions of two replicate pairs are compared with methane production of a sediment control.

The rate of methane production in acetone-spiked sediment is comparable to the methane production of sediment control. The overall methane production did not increase due to the addition of 20 mg/L of acetone. This indication means that methane production with addition of acetone is the same as background condition (natural response without addition of acetone). The level of substrate addition is therefore not comparable with the concentration of bioavailable natural organic matter in sediments, hence, the methane production caused by acetone addition is negligible. An approximate ten-day acclimation period for acetone-spiked sediment was observed and indicated that the incubated microcosms were adapting to the loading of acetone and enriched nutrient medium.

Phenol. Methane production of indigenous microorganisms by metabolizing phenol plus natural organic matter (NOM) in sediment and NOM alone (sediment control) is illustrated in Figure 4.5.7. Methane production with phenol addition has longer lag phase (6 days) compared with sediment control (3 days), but the rate of methane production for both conditions were similar. Again, the lag phase is for acclimation of bacteria to the loading of phenol and enriched nutrient medium.

The degradation of phenol versus time is detected in aqueous and sediment phases using GC-FID. The uptake of phenol by the anaerobic bioreactor is compared with total recovery of abiotic system in which the sediment is sterilized and phenol is added under the same condition as biotic system. The experimental results are shown in Figure 4.5.8 and 4.5.9. The overall phenol recovery under abiotic conditions ranged from 80 to 100% and no degradation was observed under abiotic conditions. The phenol extracted from aqueous-supernatant and sediment phases in biotic samples decreased with time, and the uptake of phenol in aqueous supernatant phase is faster than phenol in sediment phase. In 27 days of period, phenol in aqueous and sediment phases was depleted by 40% and 15%, respectively, resulting in an apparent removal of 55% for phenol. Phenol therefore was apparently degraded in the sediment system and methane production was equivalent to the control due to the low net input of substrate carbon relative to sediment carbon.

Benzene. Figure 4.5.10 shows methane production of active sediment uptaking benzene plus NOM and NOM alone (sediment control). No acclimation period was observed for methane production with benzene addition and the production rate is almost parallel to the sediment control. The lack of an acclimation period can be explained that the indigenous microcosms were acclimated to benzene and they could respire without delay under favorable conditions (enrichment of nutrients).

The fate of benzene using SED-03* sediment under methanogenic conditions is illustrated in Figures 4.5.11 and 4.5.12. Benzene is extracted from aqueous supernatant and sediment phases. Benzene extracted from abiotic controls had constant recovery rates (85% to 100%) and no disappearance of benzene was detected. The recovery of

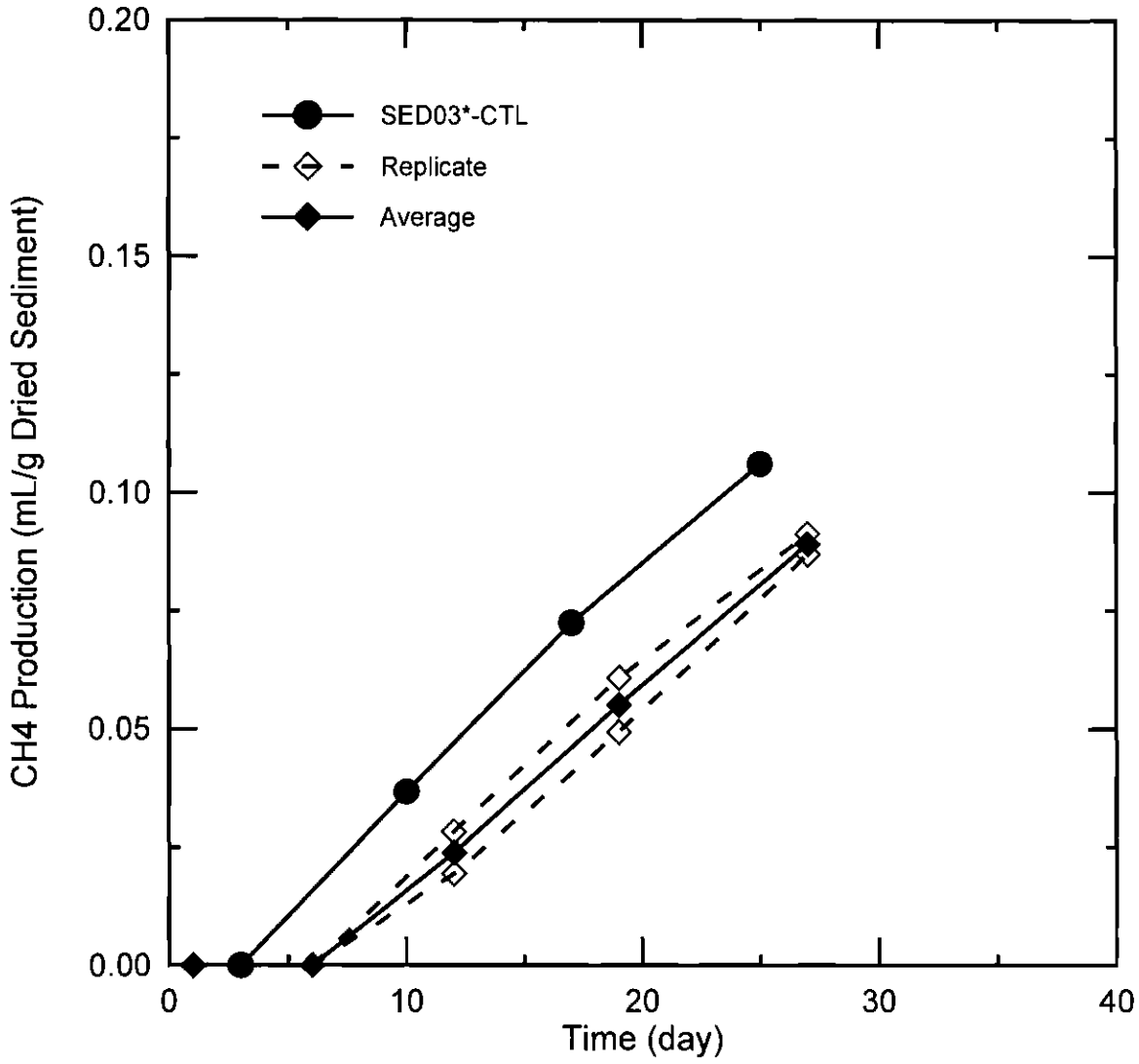


Figure 4.5.7. CH_4 production of phenol (21 mg/L) using NA-RAFB-0996-SED-03 sediment at concentration of 50.47 g/L.

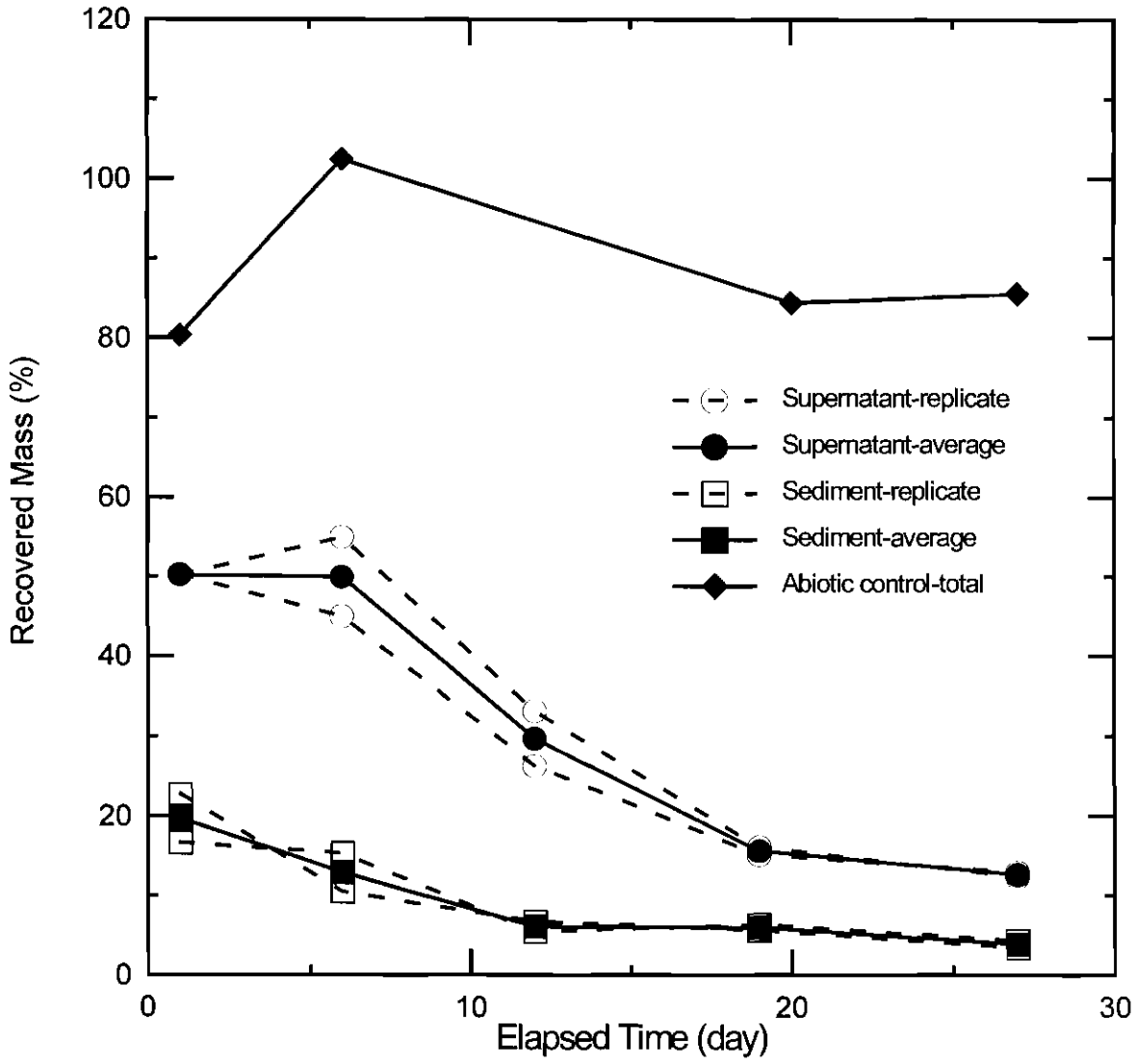


Figure 4.5.8. Recovery of phenol (21 mg/L) using NA-RAFB-0996-SED-03 sediment under methanogenic conditions.

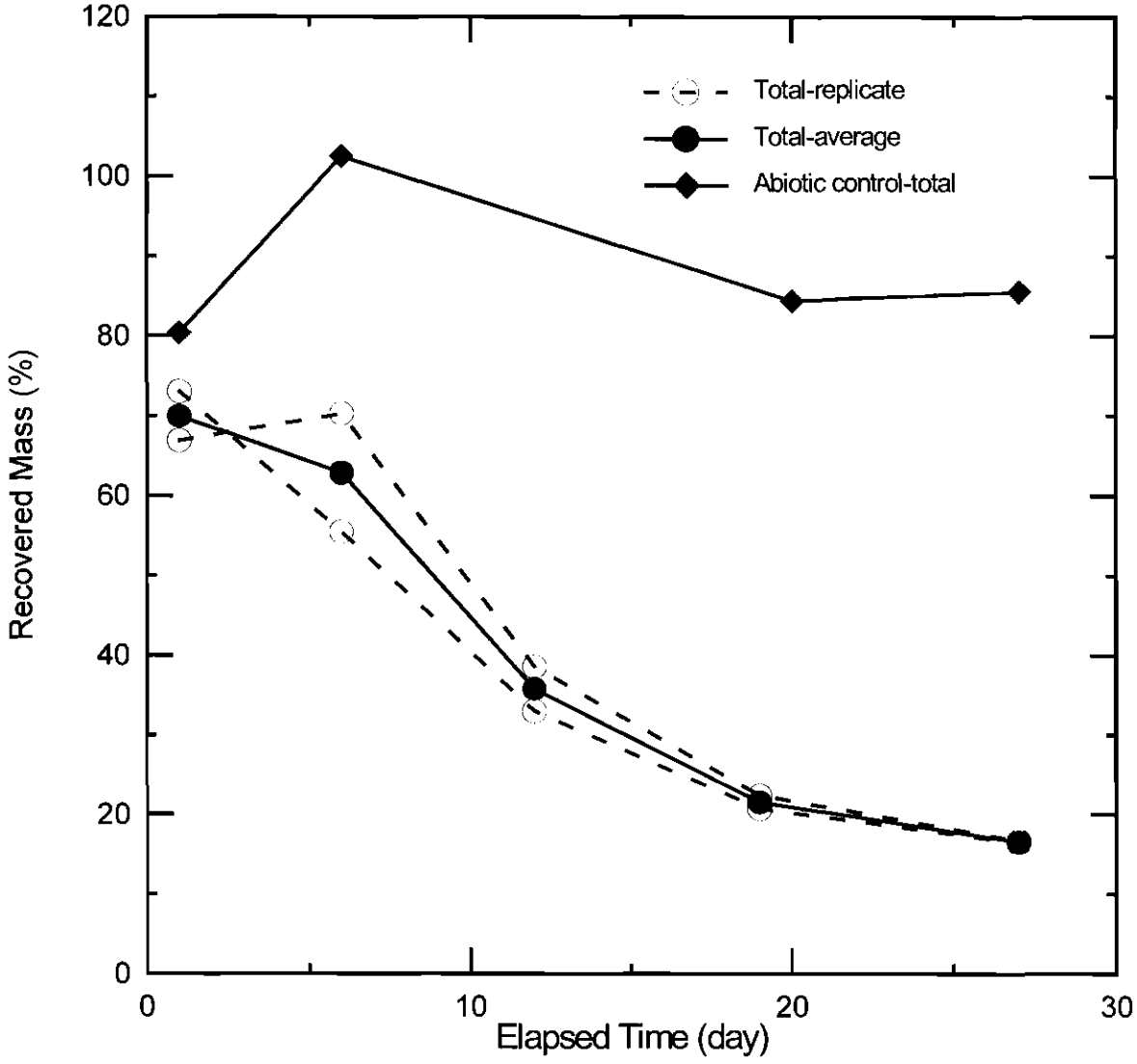


Figure 4.5.9. Apparent removal of phenol using NA-RAFB-0996-SED-03 sediment under methanogenic conditions.

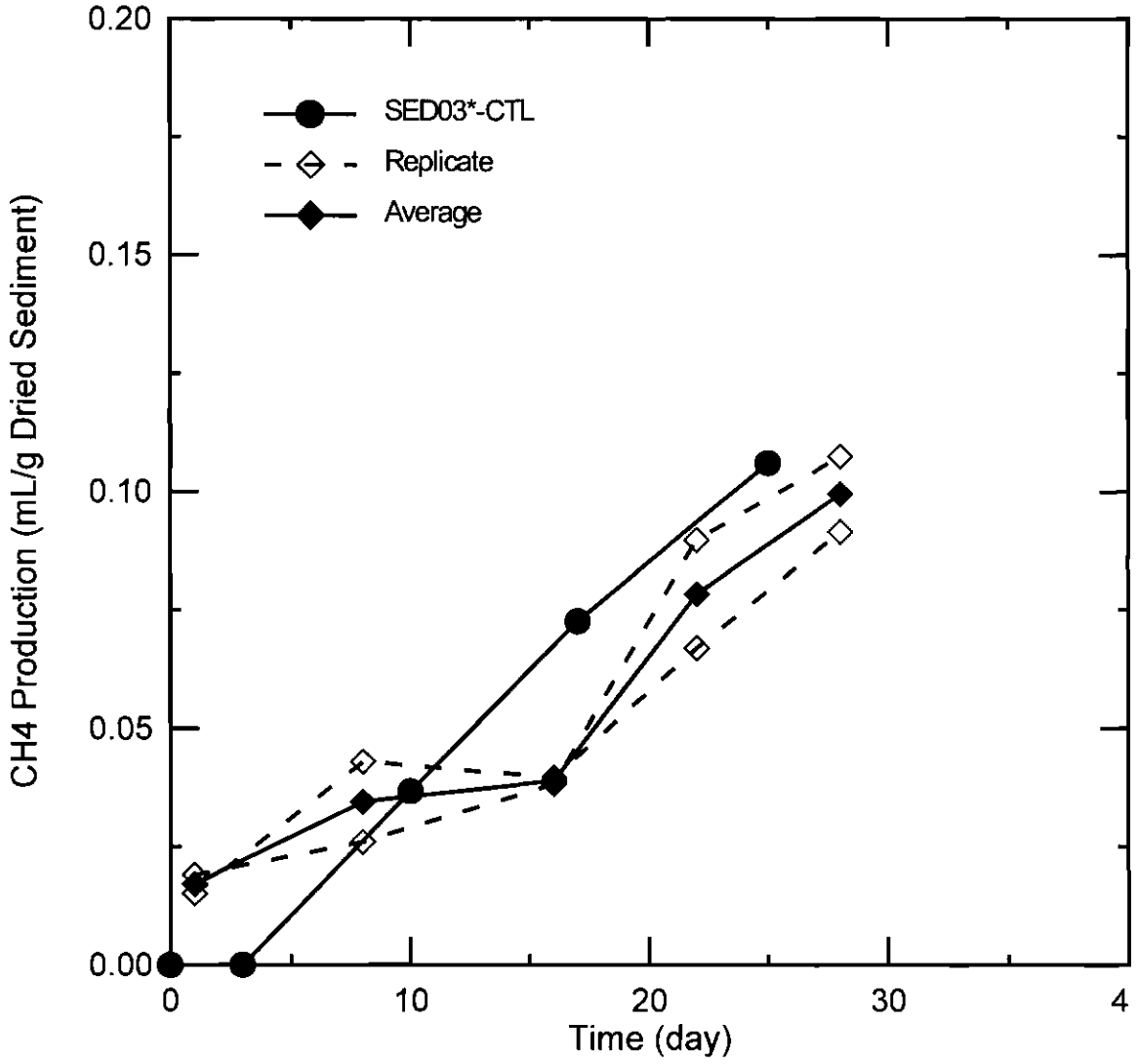


Figure 4.5.10. CH_4 production of benzene using NA-RAFB-0996-SED-03 sediment under methanogenic conditions. Initial benzene concentration was 14.73 mg/L and sediment concentration was 50.33g/L.

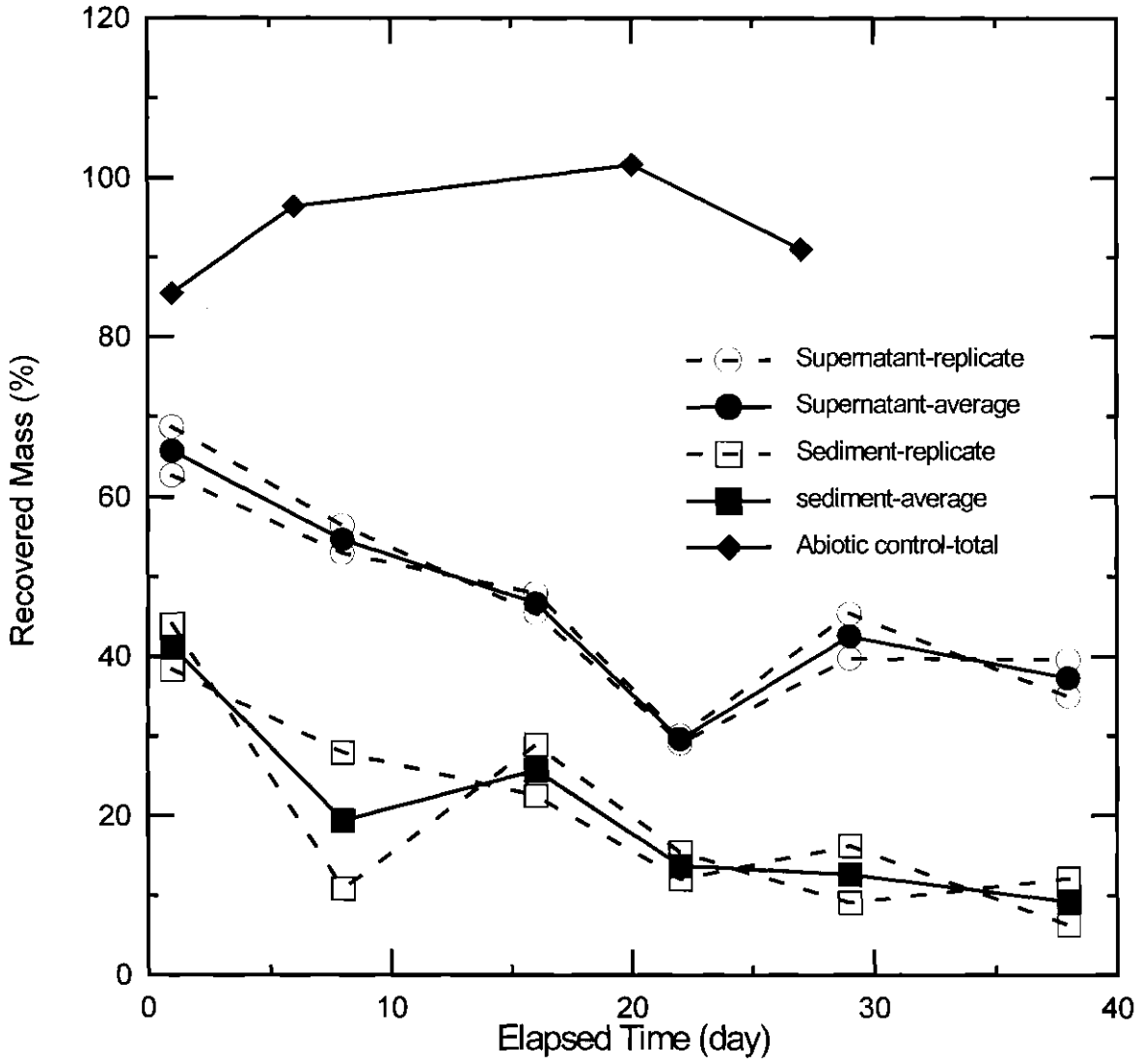


Figure 4.5.11. Fate of benzene using NA-RAFB-0996-SED-03 sediment under methanogenic conditions. Benzene, 14.73 mg/L; sediment ratio, 50 g (dried weight)/L.

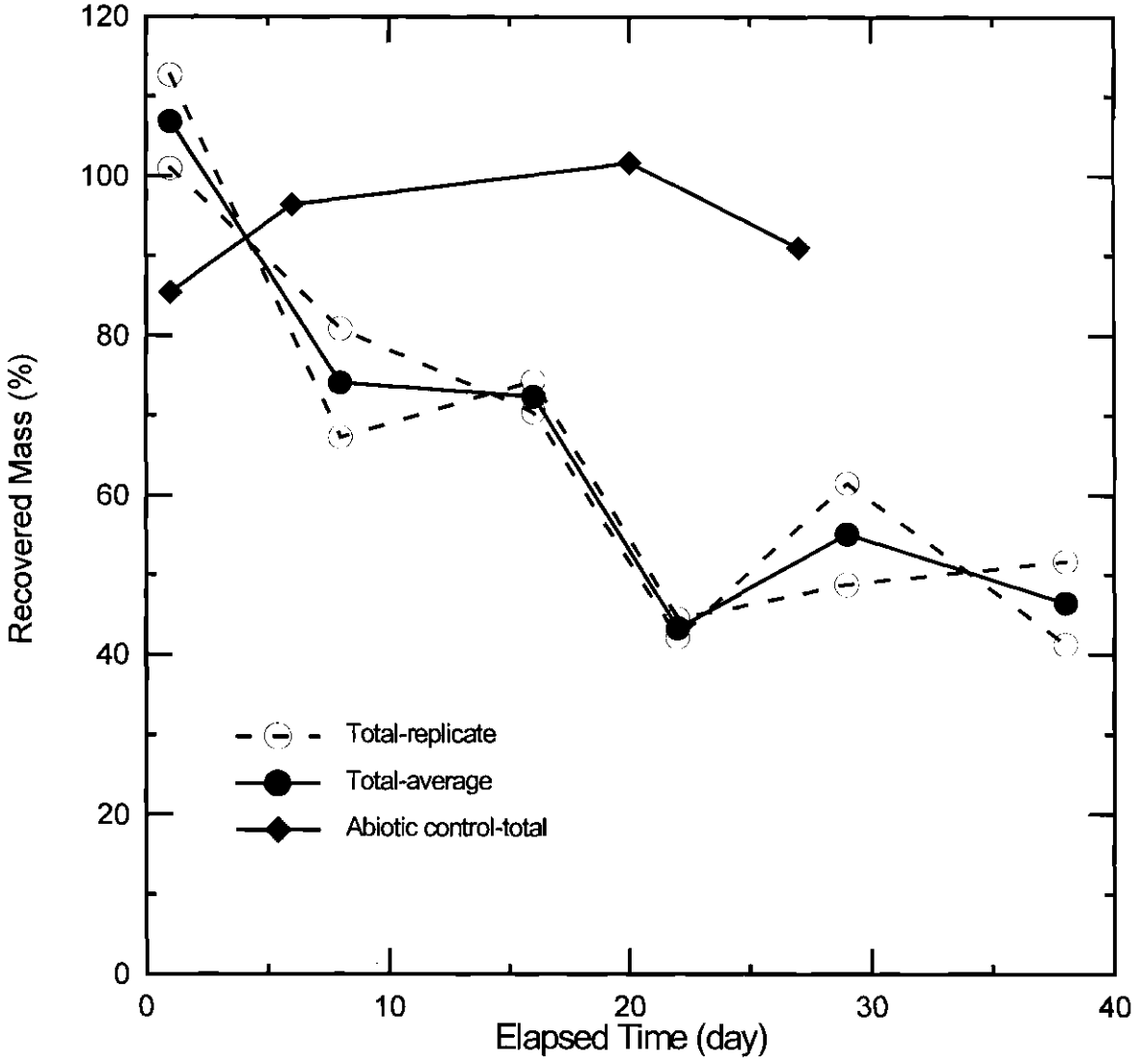


Figure 4.5.12. Overall fate of benzene using NA-RAFB-0996-SED-03 sediment under methanogenic conditions. Benzene, 14.73 mg/L; sediment ratio, 50 g (dried weight)/L.

benzene in aqueous phase started with 65% and decreased to 35% in 38 days of period, and the recovery of benzene in sediment phase is 40% at beginning and decreased to 10% after 38 days. Note that benzene declined equally in the aqueous and sediment phases and this can involve biotransformation, partitioning and re-equilibrium of benzene in aqueous and sediment phases. The data in Figures 4.5.11 and 4.5.12 indicate that benzene was not recovered completely (compared to abiotic control) over a 38-day period. While mineralization did not occur, there were processes involved that resulted in complexation, transformation conjugation or irreversible adsorption and an apparent decrease in benzene in this high-carbon sediment.

Consistent with the measurement of methane production, no acclimation of benzene is observed in bioactive wetland sediment. There was no confirmation that benzene was not degraded in wetland sediments under anaerobic conditions.

Chlorobenzene. Methane production in the presence of chlorobenzene shows no acclimation period, but the rate of methane production is slower than sediment control which has no addition of chlorobenzene (Figure 4.5.13). This inhibitory effect of chlorobenzene may affect a portion of the microbial population which is not acclimated to chlorobenzene loading, and therefore inhibit the affected microbial population to produce methane.

The partitioning of chlorobenzene into the sediment phase is quite active based on the observation of chlorobenzene recoveries from sediment phase (Figure 4.5.14. and Figure 4.5.15.). The equilibration between the aqueous and sediment phases takes place over a 40-day period and could also include biotransformation of chlorobenzene. The inhibition of methane production however indicates a negative biological impact much less an enhanced production of methane (or CO_2) from chlorobenzene. The biological fate of chlorobenzene is uncertain but biodegradation is not confirmed or indicated.

1,4-Dichlorobenzene. No methane production was detected for sediments with 1,4-dichlorobenzene (Figure 4.5.16). This inhibition is significant for unacclimated indigenous microcosms. This result is consistent with the results of GC-analyses. No degradation is observed on extracts of 1,4-dichlorobenzene in either aqueous supernatant phase nor sediment phase (Figure 4.5.17 and Figure 4.5.18.). No biodegradation of 1,4-dichlorobenzene can therefore be concluded.

TCE. Methane was detected at 0.03 mL/g level on the second day of the TCE experiment (Figure 4.5.19). Methane production increased only slightly to approximately 0.06 mL/g over the 28-day period. At best, there is less than 0.03 mL/g of methane produced during 28 days of exposure. This response is best interpreted as a significantly

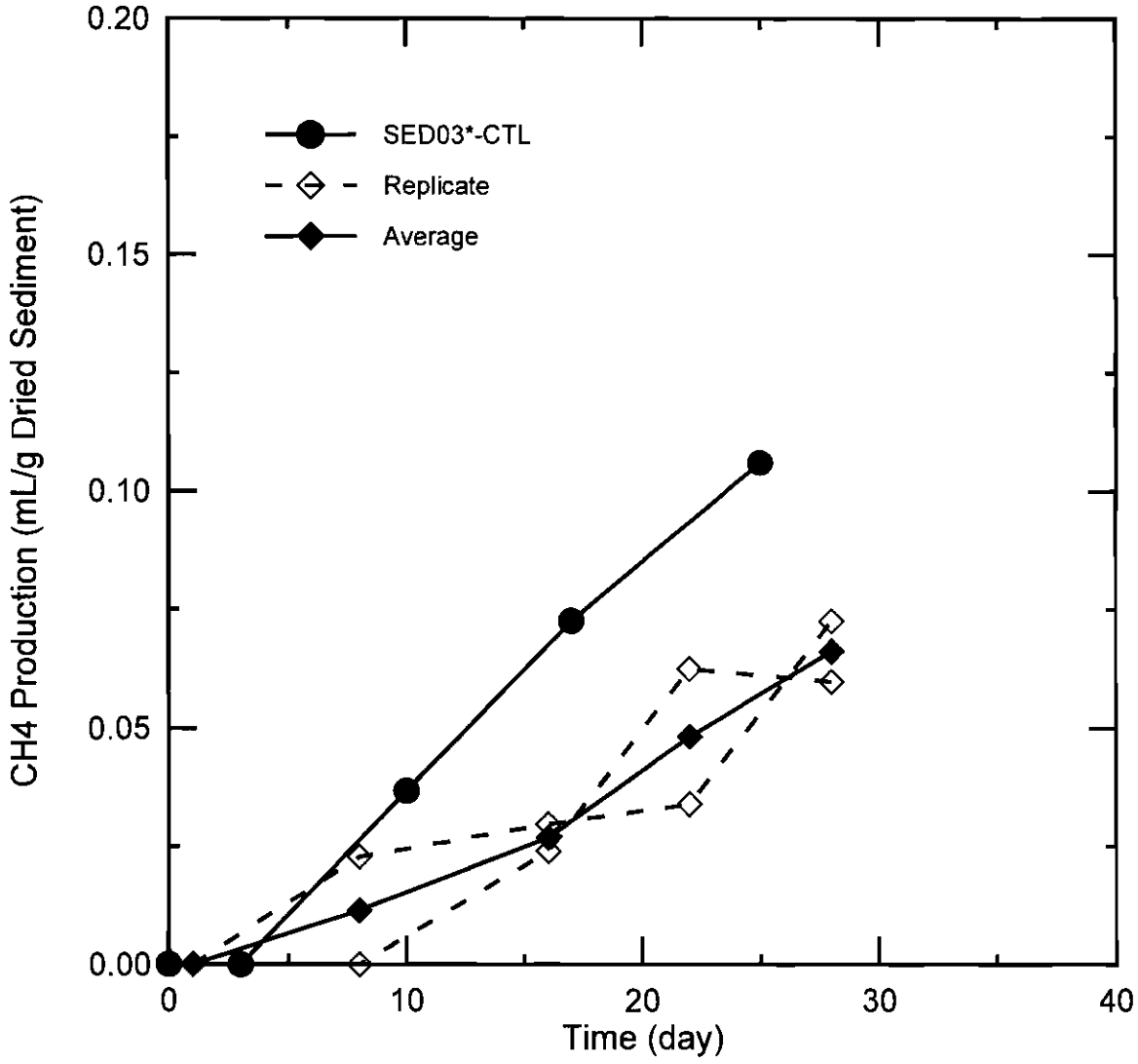


Figure 4.5.13. CH_4 production in presence of chlorobenzene (10.7 mg/L) using NA-RAFB-0996-SED-03 sediment at a concentration 50.44 g/L.

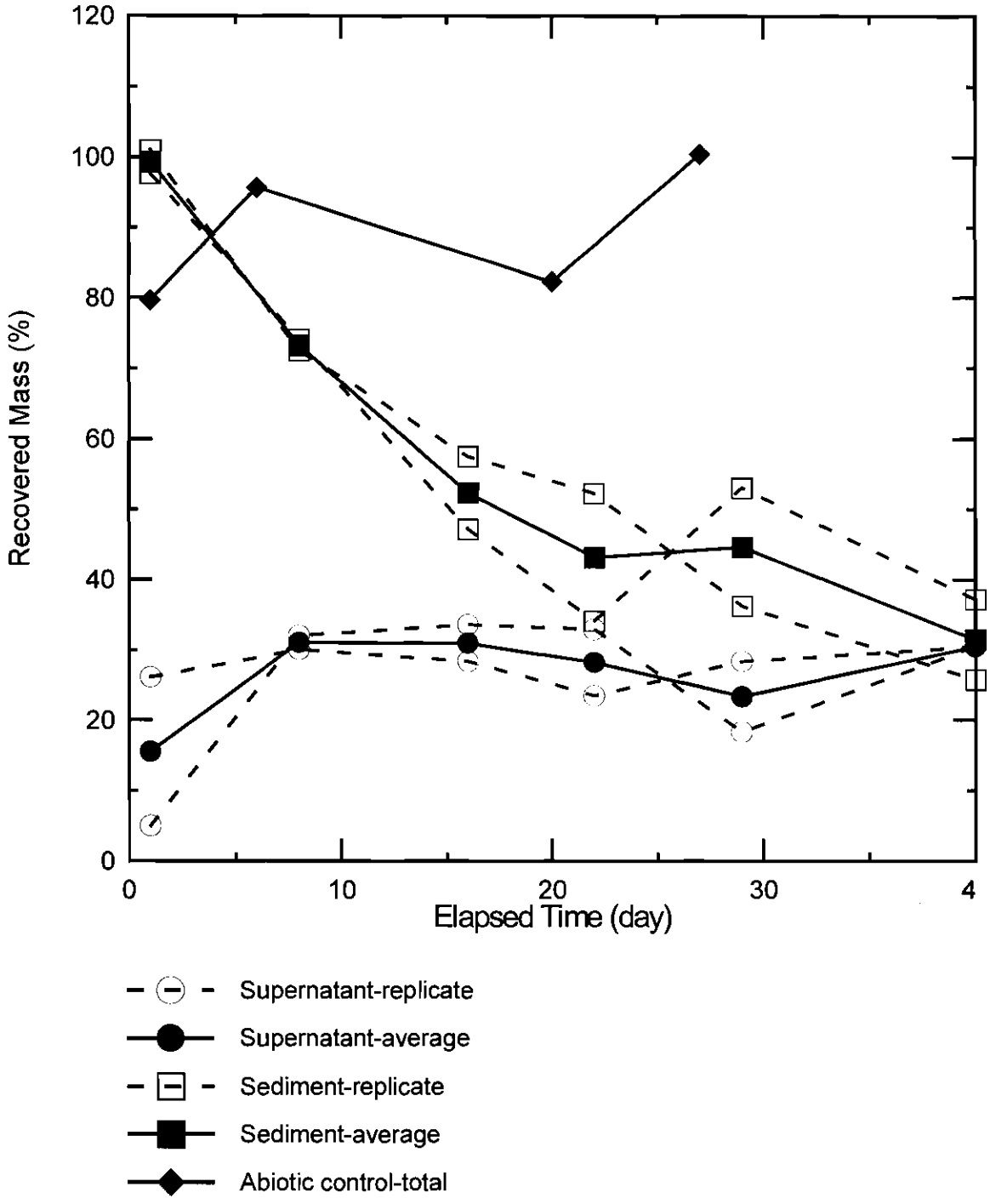


Figure 4.5.14. Fate of chlorobenzene using NA-RAFB-0996-SED-03 sediment under methanogenic conditions. Initial chlorobenzene concentration was 10.70 mg/L and sediment concentration was 50.44 g/L.

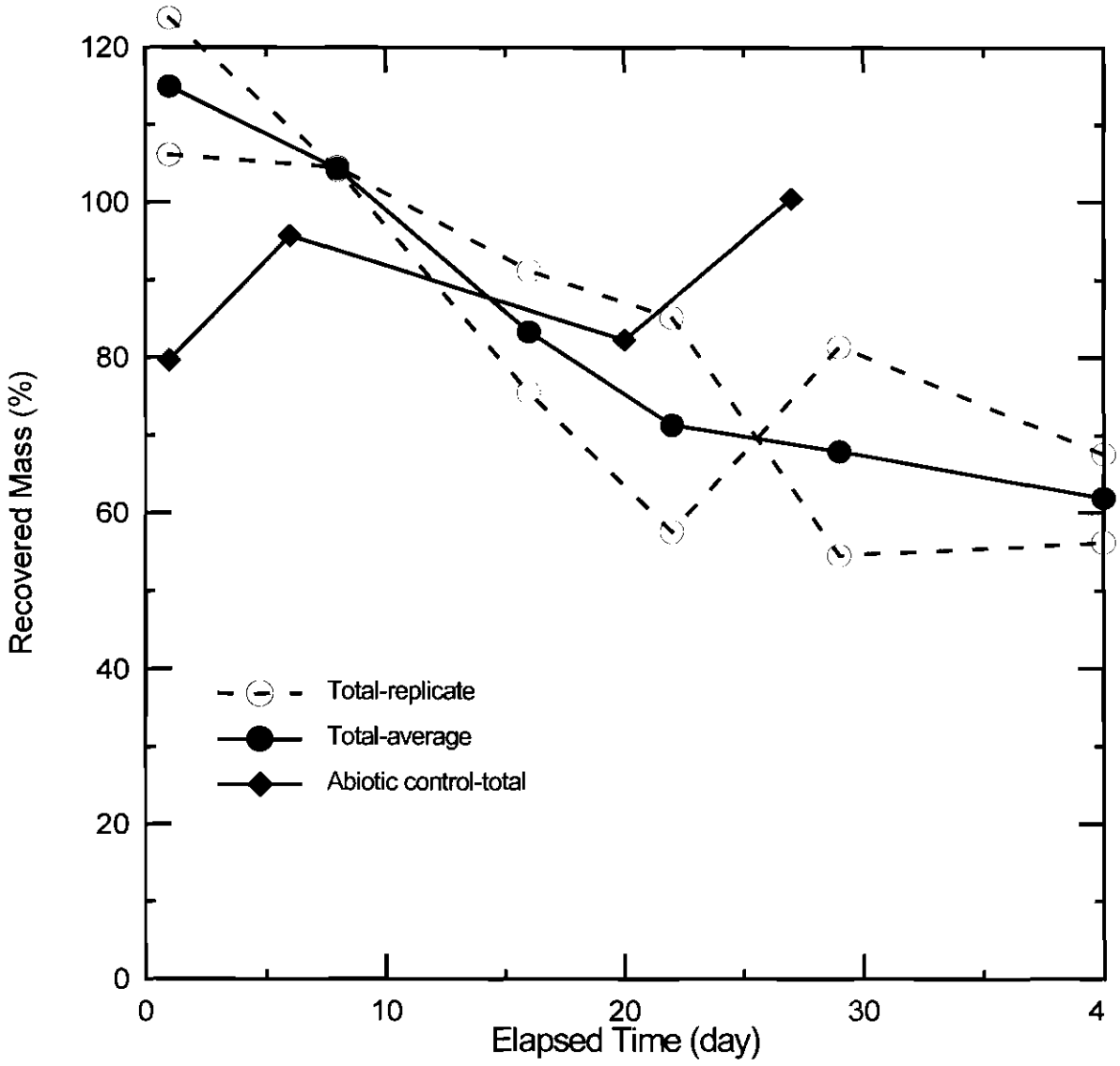


Figure 4.5.15. Overall biodegradation of chlorobenzene using NA-RAFB-0996-SED-03 sediment under methanogenic conditions. Chlorobenzene, 10.70 mg/L; sediment ratio, 50.44 g (dried weight)/L.

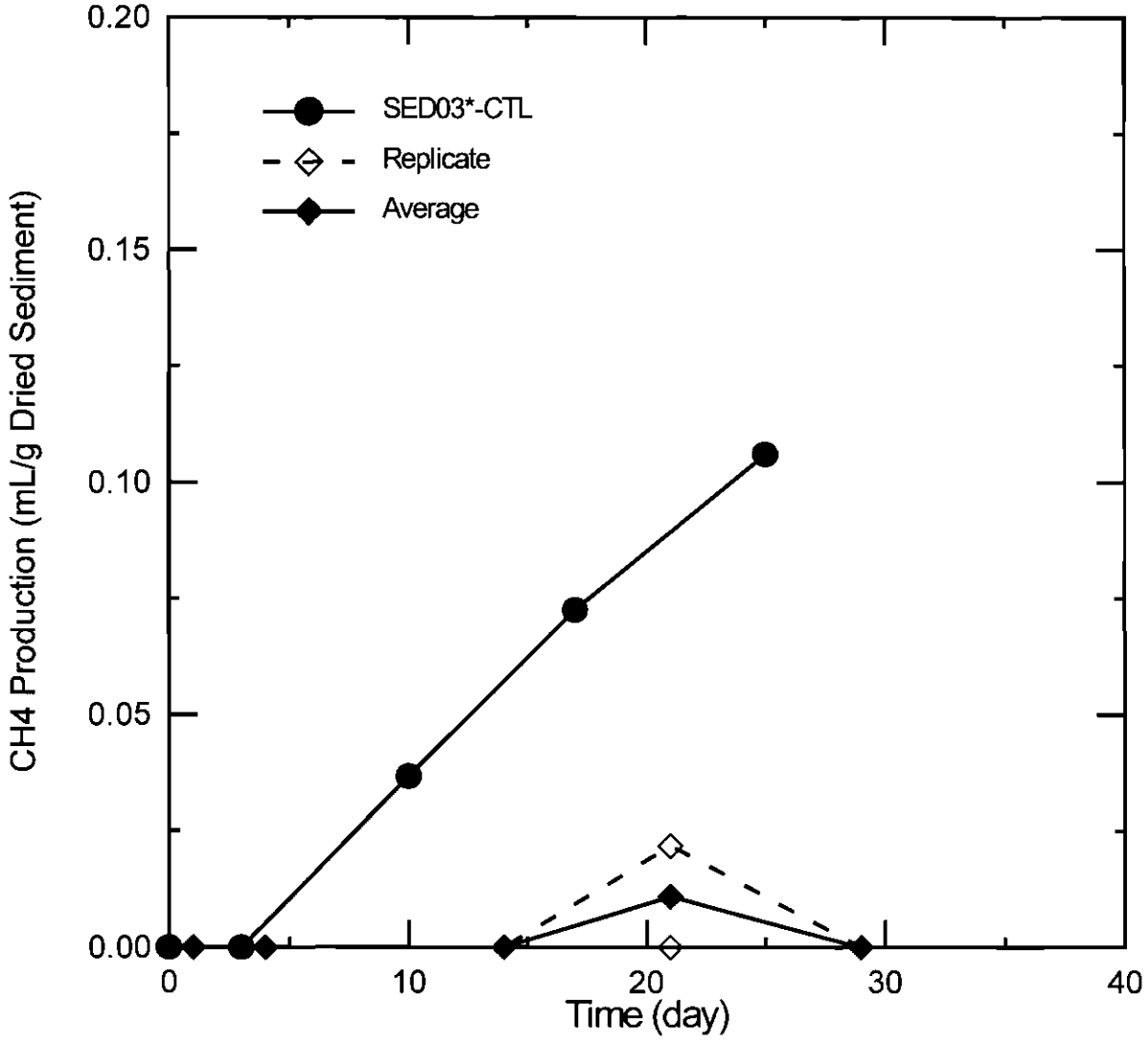


Figure 4.5.16. CH_4 production of 1,4-dichlorobenzene using NA-RAFB-0996-SED-03 sediment under methanogenic conditions. Initial 1,4-dichlorobenzene concentration was 13.11 mg/L and sediment concentration was 52.02 g /L.

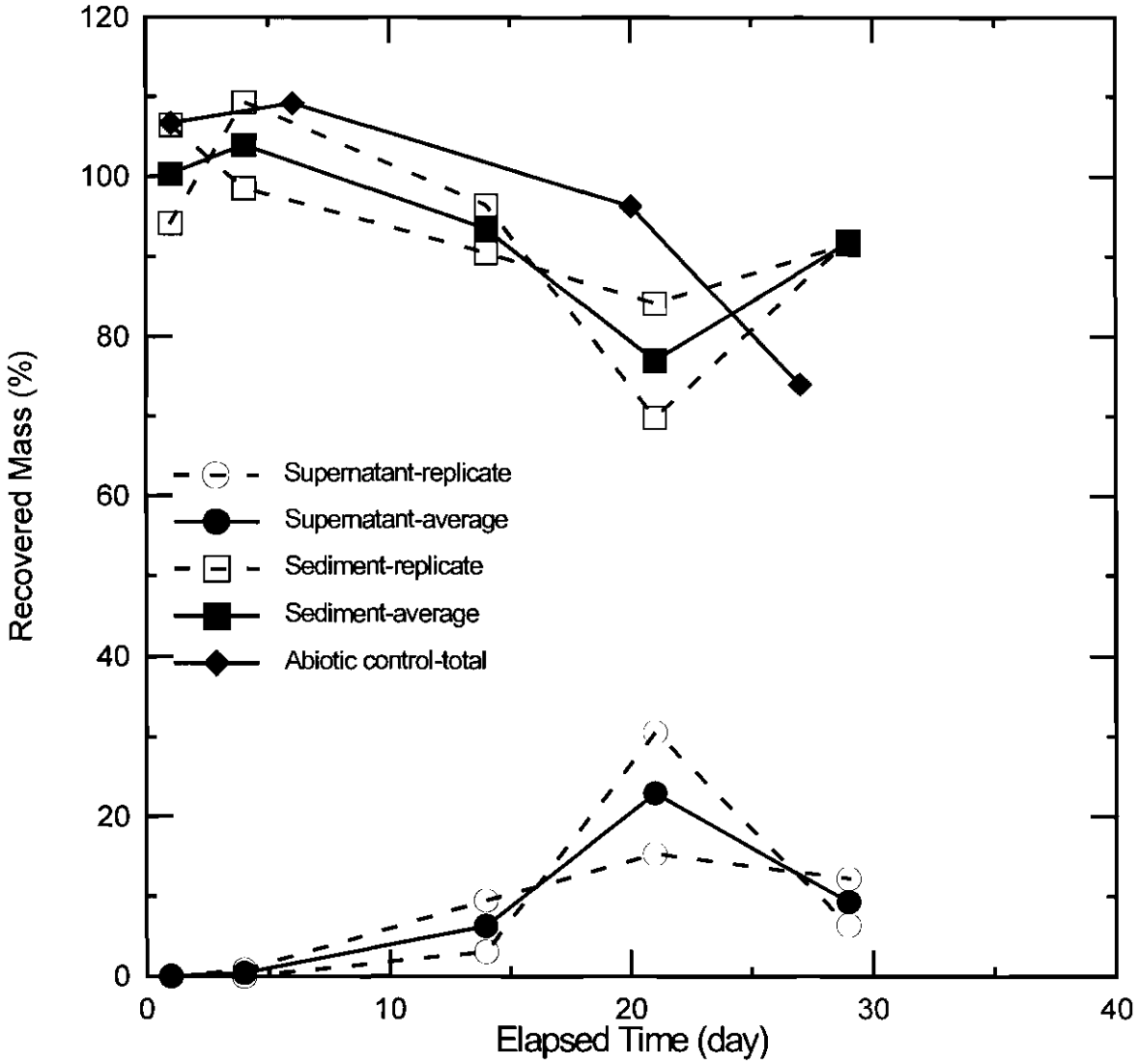


Figure 4.5.17. Biodegradation of 1,4-dichlorobenzene using NA-RAFB-0996-SED-03 sediment under methanogenic conditions. 1,4-Dichlorobenzene, 13.11 mg/L; sediment ratio, 52.02 g (dried weight)/L.

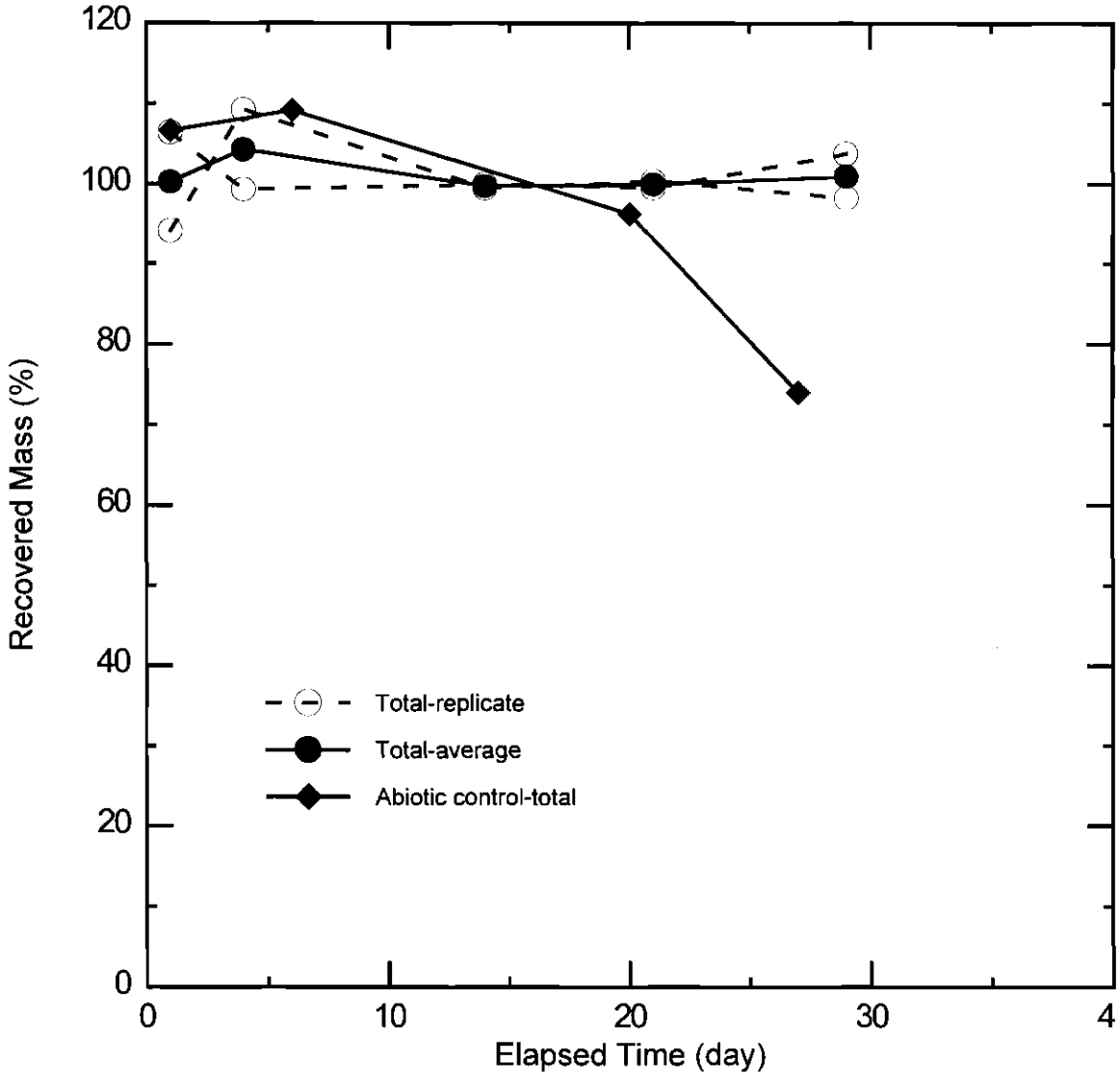


Figure 4.5.18. Overall disappearance of 1,4-dichlorobenzene using NA-RAFB-0996-SED-03 sediment under methanogenic conditions.

reduced or inhibited production of methane. If normalized to initial methane levels on day 2, the minimal production of methane over the 28-day period would indicate near-total inhibition of methanorganic activity in the sediment. TCE is therefore an apparent toxin to the indigenous methanogens in the sediment.

TCE partitioning in the serum-bottle system is presented in Figures 4.5.20 and 4.5.21. TCE was significantly reduced over the 28-day period in both the aqueous and sediment phases. This response is attributable to: strong partitioning into the organic- and mineral-phase of the sediment and transformation to products (e.g., DCE, vinyl chloride, ethylene). These transformation products were not monitored and could not be confirmed. Therefore, TCE was inhibitory to biological methanogenesis and was potentially transformed in the 28-day period of exposure.

Kinetic Summary. In Table 4.5.1, the zero-order rates of methane production are indicated. Methane production for acetone has the highest kinetic rate, then phenol, benzene, dichlorobenzene and TCE. No methane production was indicated for 1,4-dichlorobenzene.

The overall data developed under anaerobic indicated that acetone and phenol are biodegradable in Robins AFB wetland sediments. TCE, chlorobenzene, dichlorobenzene and benzene were not degraded. TCE and 1,4-dichlorobenzene inhibited methanogenic activity.

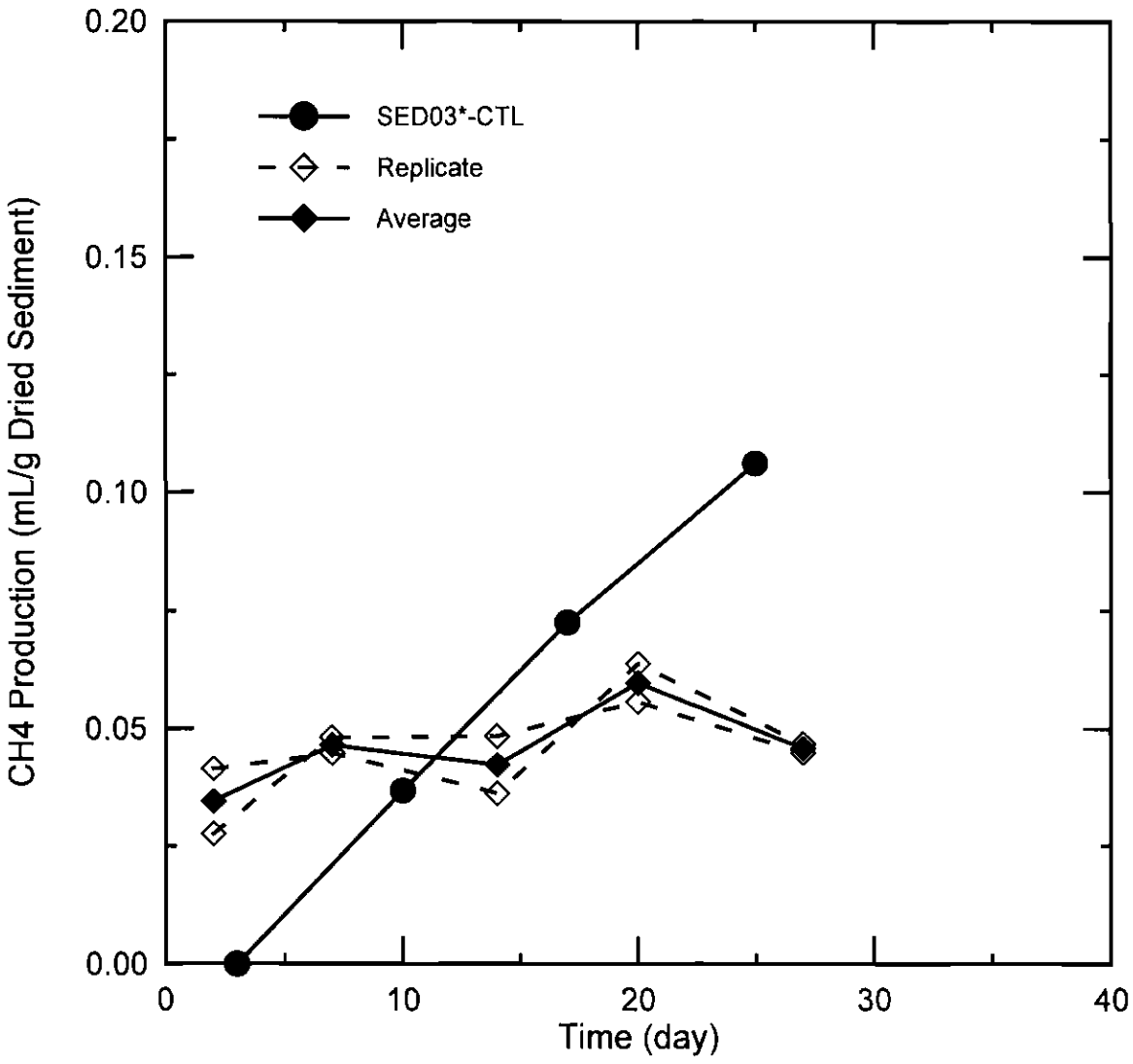


Figure 4.5.19. CH_4 production with TCE (14.07 mg/L) using NA-RAFB-0996-SED-03 sediment at 51.97 g/L.

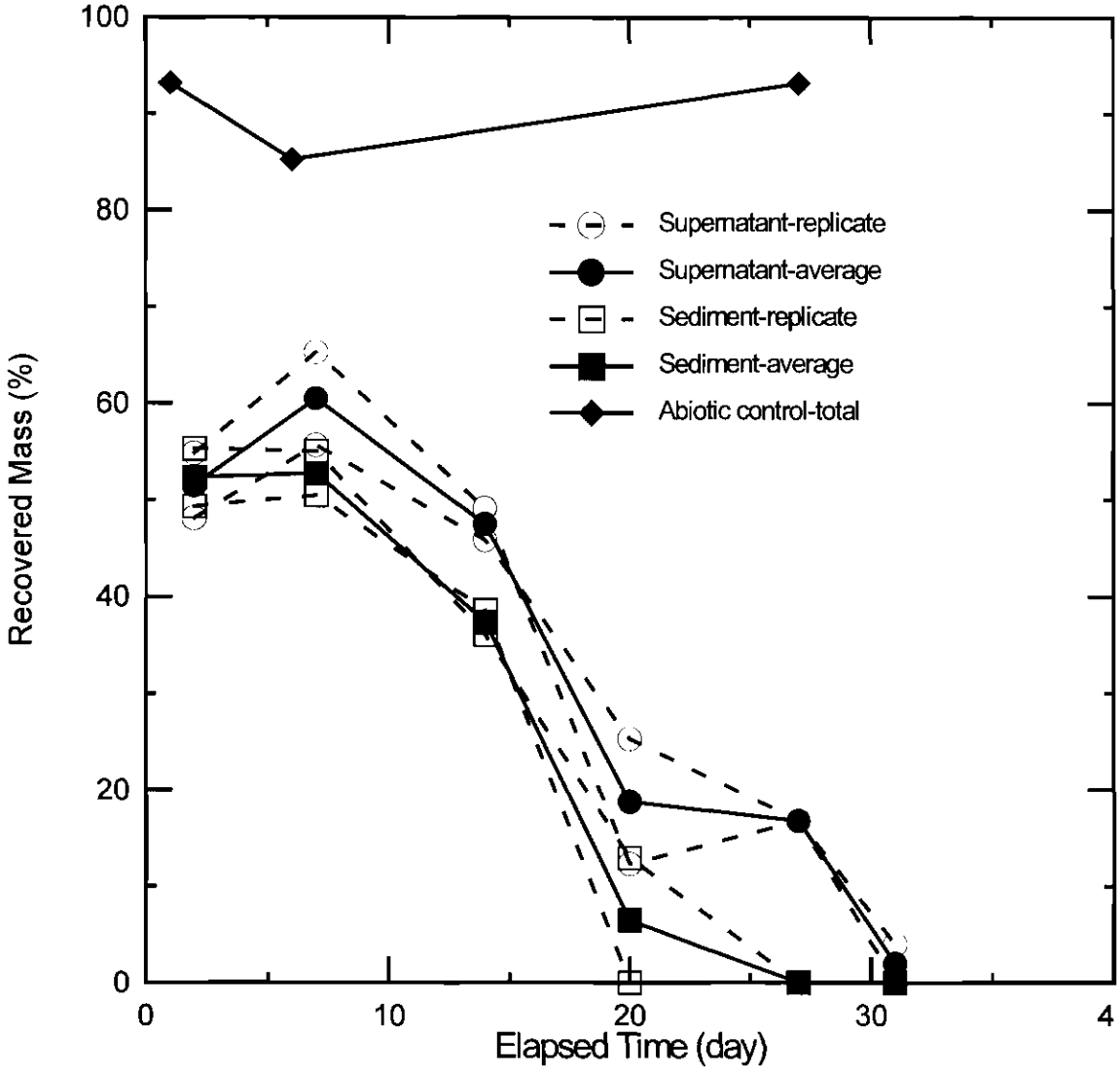


Figure 4.5.20. Partitioning of TCE in aqueous- and sediment-phases of NA-RAFB-0996-SED-03 sediment under methanogenic conditions.

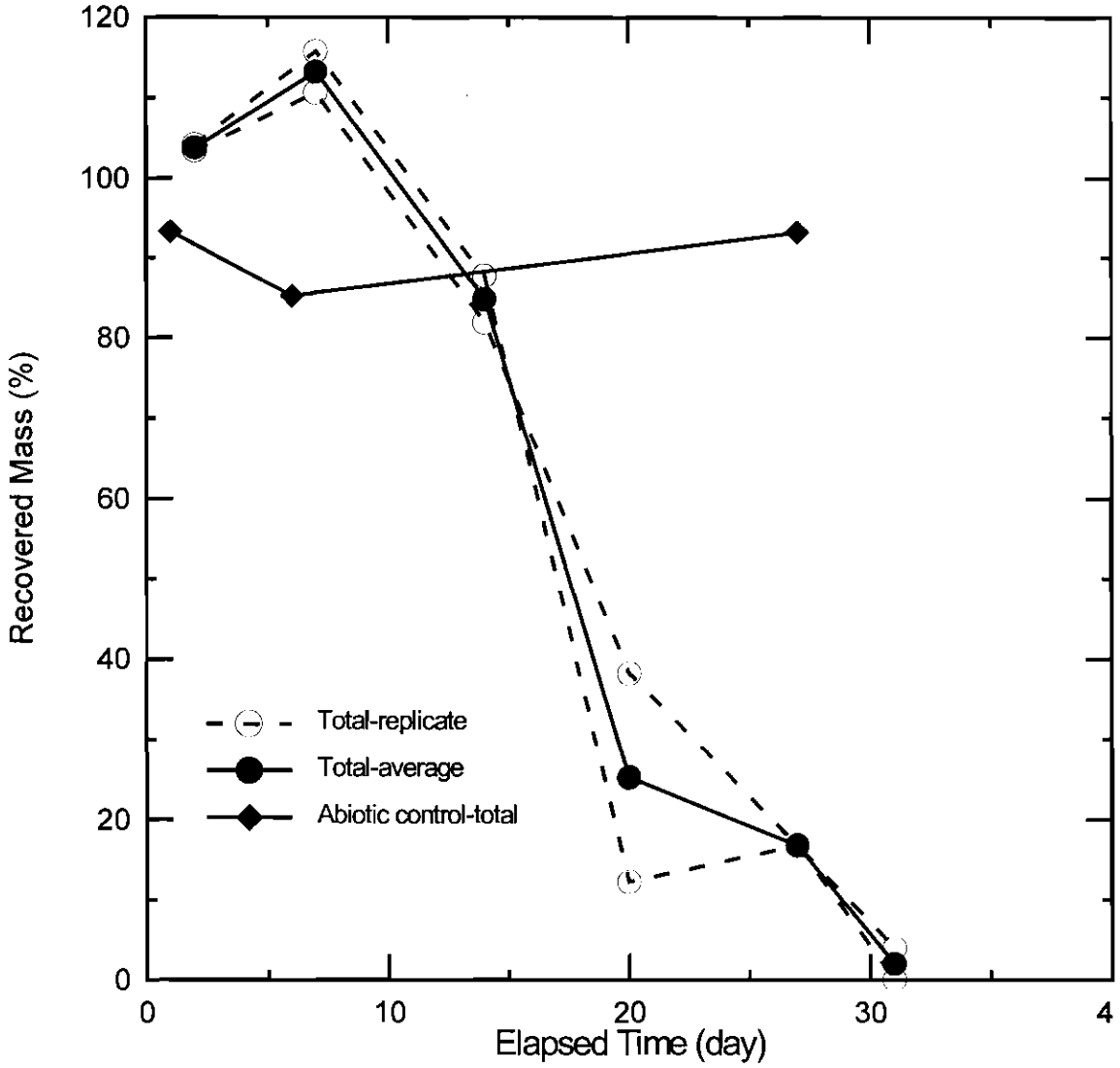


Figure 4.5.21. Overall biodegradation of TCE using NA-RAFB-0996-SED-03 sediment under methanogenic conditions. TCE, 14.07 mg/L; sediment ratio, 51.97 g (dried weight)/L.

Zero Order Kinetic Data Target Contaminants	Slurry, M (g/L)	Zero Order k Value (mL/(g-d))	RSQ
NA-RAFB-0996-S03			
Acetone	52.00	0.0046	0.93
Phenol	50.47	0.0034	0.95
Benzene	50.33	0.0025	0.91
Chlorobenzene	50.50	0.0019	0.85
1,4-Dichlorobenzene	52.08	0.0000	--
TCE	51.97	0.00152	0.72

NOTE: The control reactor in this study had a zero order methane production rate, k, of 0.0046 mL/(g-d).

Table 4.5.1. Kinetic summary of methane production of specific site contaminants

4.6 Sediment Column Investigation

The bioremediation of a wide variety of site contaminants in a wetland bioslurry under both aerobic and anaerobic environments needs to be understood in continuous-flow column system. The response of such a continuous-flow system can be used to simulate conditions. The complexity of the wetland systems at Robins AFB and the direct dependence on rooted plants in defining the sediment systems make it impossible to simulate the sediment systems within this project. Nonetheless preliminary sediment columns were investigated to establish a potential path forward to a necessary *in situ* field-simulation of natural attenuation.

An initial column study was designed using an up-flow mode with 260-cm hydraulic head to measure the conductivity of sediments packed in a glass column (30-cm length × 2.5-cm ID). The sediment characteristics were best described as similar to those of fine clay particles and a slow (~ 1mL/d) and unstable flow was collected with a head of 260 cm. Some measured sediment characteristics were included in Table 4.6.1.

Parameter	Value
Sediment(%)/Sand(%)	100%/0%
Flow, Q	0.0714 mL/hr
Conductivity, K	3.72×10^{-7} cm/hr
Permeability, k	3.4×10^{-12} cm^2
Bulk Density, ρ_b	1.21
Porosity, n	0.54
Column Volume	117.81 cm^3
Pore Volume	79.8 cm^3

Table 4.6.1. Characteristics of Wetland Sediment Column

To address flow limitations, flow was improved by mixing sediment with sand (30-60 mesh size). Two flow tests were conducted with sand contents of 20% and 50%. The column was 30 cm long with 2.5 cm ID, with a head of 260 cm. During a long period of observation of flow measurement, the flow rates improved, especially with 50% sand addition; the overall flow was found to be unstable. The characteristics of these two sand-sediment columns are listed in Table 4.6.2.

Parameter	Value	
Sediment (%)/Sand (%)	80%/20%	50%/50%
Flow, Q	0.37 mL/hr	2.00 mL/hr
Conductivity, K	2.4×10^{-6} cm/s	1.3×10^{-5} cm/s
Permeability, k	2.19×10^{-11} cm^2	1.19×10^{-10} cm^2
Bulk Density, ρ_b	1.32	1.87
Porosity, n	0.50	0.29
Column Volume	117.81 cm^3	117.81 cm^3
Pore Volume	74 cm^3	43.3 cm^3

Table 4.6.2. Characteristics of Wetland Sediment Columns with Addition of Sand

To address unstable-flow issues, the influent flow to the column was pumped with a peristaltic pump at the rate of 0.3 mL/hr through a 50% sand/sediment media with a 26-cm column length. The first stage of the study of these reconstructed columns was a tracer study. Bromide was used as a tracer because it does not exist in background waters and does not react to any appreciable extent with other ions in solution or the porous medium. Residence-time-distribution analysis is carried out by injecting bromide into the liquid as it enters the column and monitoring the effluent tracer concentration.

In this study, two soil columns (16-cm long×25mm diameter) were constructed. One 100% sand column was packed as a reference column and was compared to a column with 60% sand and 40% wetland sediment (i.e., NA-RAFB-0996-SED-03-02). The packing materials were wetted before packing with groundwater collected from a Robins AFB groundwater well. The glass columns had 20 μm Teflon filters placed at both ends of the columns to prevent soil depositing in the feed and effluent lines.

Solute breakthrough curves were determined with bromide injection. Sodium bromide (0.1M) was prepared in filtered groundwater and was pumped from reservoirs through influent lines to two columns. The bromide solution is being purged with nitrogen continuously and the experiment was run at room temperature (~22^{0}C). Columns were operated in saturated up-flow mode. Flow through each column was held constant at 0.3 mL/hr using a peristaltic pump on the influent line. Column effluents were collected in volumetric syringes to precisely measure effluent flow rates. The effluent solution was collected twice a day and the breakthrough of bromide ion was monitored (with ion chromatography).

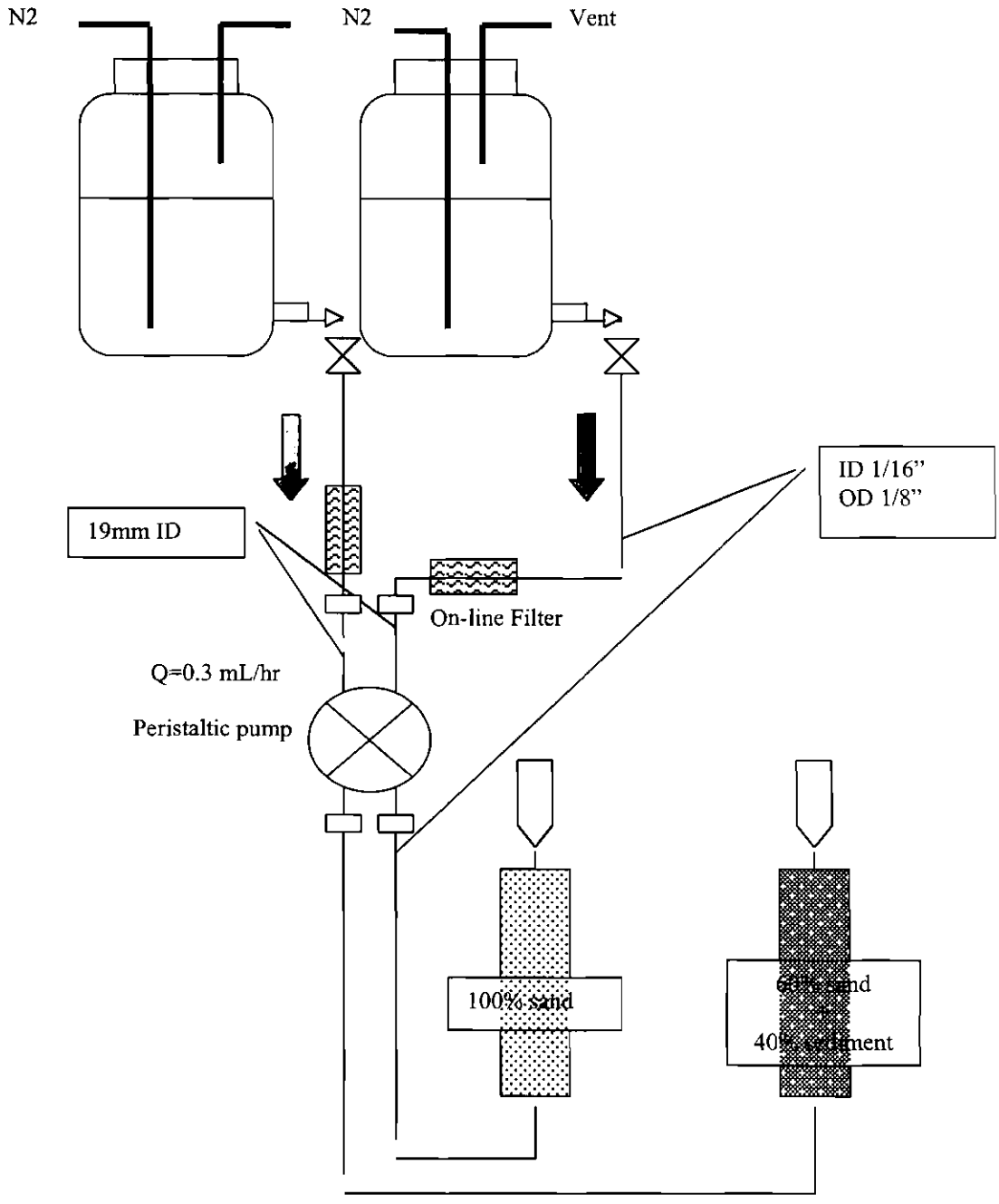


Figure 4.6.1. Schematic diagram of column study

In this study, sodium bromide (0.1M) flowed through the columns at 0.3 mL/hr for 300 hr and 480 hr, respectively for the 100% and 60% sand columns.

For the 100% sand column, bromide concentration in the effluent line increased exponentially and reached plateau saturation phase (Figure 4.6.2) in 200 hr. The plateau phase remained for another 100 hr until the feeding of bromide was terminated. Once the feeding of bromide was terminated, it took 30 hr to start the decay of the bromide and the tailing lasted approximately 1000 hr. This asymetric bell shape reveals the retardation of bromide flow.

Since the wetland sediment in the 60%/40% column had been identified as fine clay particles, a longer time to breakthrough was expected. From the measurement of effluent bromide concentration, bromide in the sediment column took 300 hr to reach a plateau saturation phase, and the duration was 100 hours longer than the sand column (Figure 4.6.2). The bromide feeding was terminated at 480 hr and the tailing of the breakthrough curve lasted for another 300 hr.

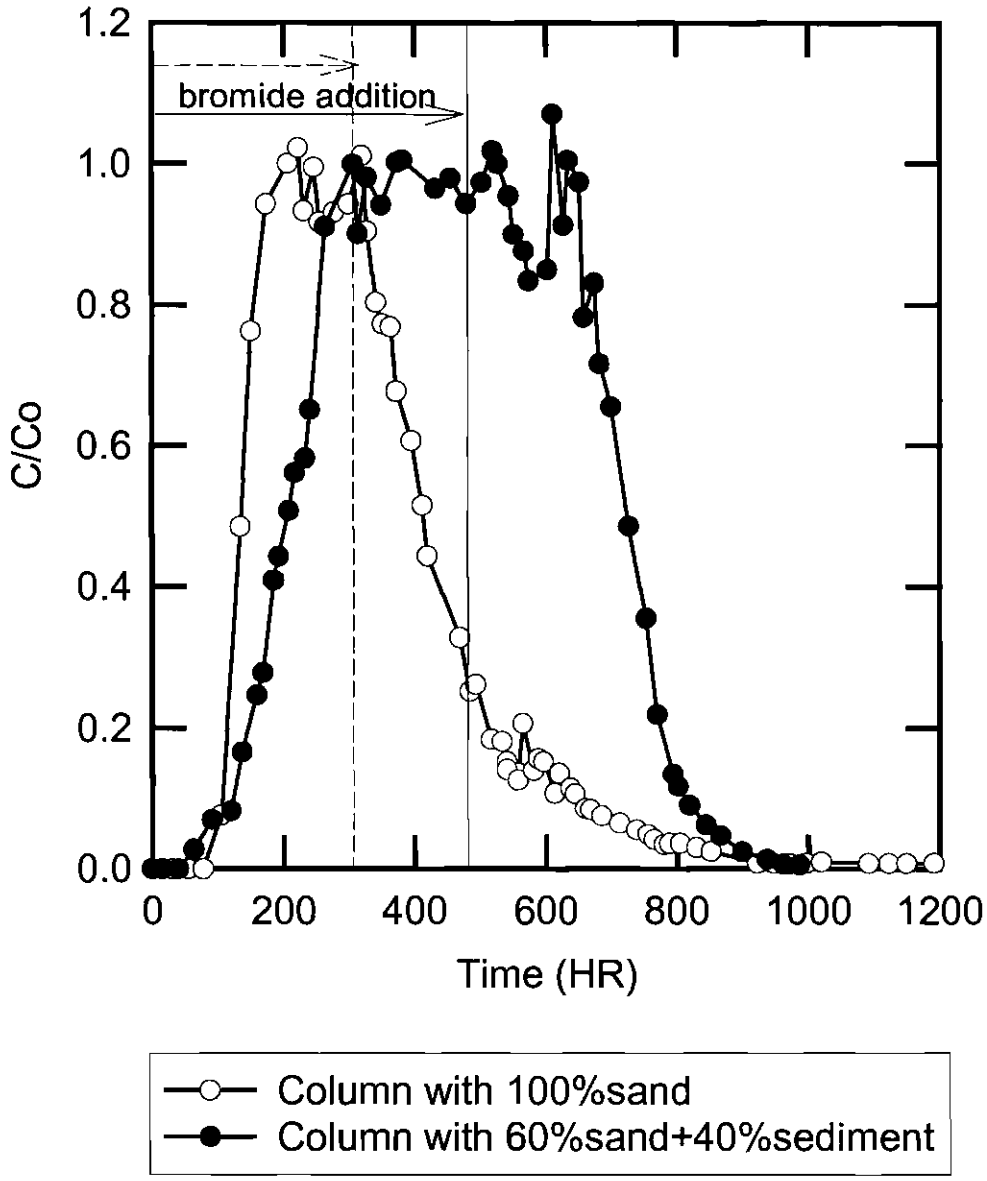


Figure 4.6.2. Bromide concentration collected from effluent line for 100% and 60% sand columns.

Site Contaminants in the Wetland Sediment Column. In order to investigate the transport and degradation of site contaminants, two sand/sediment columns were conducted in parallel. One of these columns had influent groundwater purged with N_2 (anaerobic column) and another one had influent groundwater in equilibrium with air (aerobic column). Both columns were packed with 60% sand and 40% sediment with 10.21 mL/pore volume (see system diagram in Figure 4.6.3). The transport of tracer and characeristics of these sand/sediment columns were as discussed previously.

The anaerobic and aerobic columns were fed with a mixture of acetone, phenol and benzene at an influent concentration of 2 mg/L for each compound. The total elapsed time for this study was 98 days at a flow of 7 mL/day and was equivalent to a total flow-through of 67 pore volumes. This was sufficient time to achieve breakthrough of non-reactive components. There was no detection of any of the influent contaminants in the effluents of either of the two columns. Note that the concentration of the influent contaminants were confirmed every 4 days. The column was therefore very effective in contaminant attenuation throughout this period and before it was necesary to terminate the column studies.

5. Conclusions

5.1. Physicochemical Characteristics of Sediment Samples

The natural nutrient status of a sediment directly impacts microbial activity and biodegradation. Whereas natural and anthroprogenic organic contaminants provide major sources of carbon and reducing power for microbial growth, mineral nutrients are essential for microbial activity. In particular, nitrogen, and to a lesser extent phosphorus, are necessary for cellular metabolism. A number of other environmental factors have direct impact on microbial activity in sediments. Water content, temperature, pH, E_h, organic content and dissolved organic content in pore waters are important in understanding and identifying environmental and/or physiological constraints that affect biodegradation. For instance, these parameters may affect an acclimation period of the microorganisms to the substrate or environment.

From the physicochemical results for collected wetland sediments (Table 4.1.3-Table 4.1.6), Robins AFB wetland sediments have slightly-acidic, high organic contents, high moisture content and low nutrient levels. Under this environment, microbial activities would be limited by low nutrient conditions, although the natural organic content available for wetland microorganisms is high. Concentrations of sulfate extracted from wetland sediments varied; conditions with, and without, sulfates impact the microbial activity and biodegradation differently. Particularly, if a methanogenic environment is imposed to a batch bioreactor, the microorganisms may be stressed by the competition of methanogenesis and sulfate-reducing reactions and become inactive under methanogenic condition.

Physicochemical characteristics of underlying clay and sand samples were evaluated. Clay and sand samples have relatively low organic content and water content. It is expected that the microbial populations are negligible.

5.2. Biological Characteristics of Sediment Samples

Wetland sediment samples are extremely heterogeneous and sampling to obtain a representative sample for evaluation is complex. Hence, wetland sediment and their associated microbial populations vary greatly (Table 4.1.1-Table 4.1.4), depending on geology, hydraulics, climate, nutrient availabilities and contamination. The ecology of the site may vary from site to site. Microbial populations under imposed aerobic and anaerobic environments and the data of total carbon collected in the laboratory (Figure 4.2.1 and Figure 4.2.2) showed no direct relationships. It illustrated that microbial populations were impacted by numerous environmental factors other than total carbon in the sediment.

5.3. General Microbial Activities

Oxygen consumption and methane production in sediment controls (without addition of any substrate or contaminant) indicated the response of indigenous microorganisms to biodegrade natural organic matter in the sediments under aerobic and anaerobic conditions, respectively. It is well understood that bacteria can only utilize natural organic matter for energy and metabolism. The dominant process to decompose natural organic matter is a biological reaction where bacteria release extracellular exoenzymes for hydrolysis of natural organic matter. Once dissolved by this hydrolysis, the organic matter becomes bioavailable and the bacteria may uptake it. The process of natural organic matter hydrolysis is a rate-limiting step in the Robins AFB. This phenomena furthermore is indicated by a zero-order uptake on natural organic matter in wetland sediments. Addition of dissolved, easily-degraded compounds may facilitate their uptake and degradation by natural microbial populations. Measurements with easily degraded compounds, e.g., acetate, phthlate, cresol, ethanol and benzoate, clearly demonstrated the high potential for degradation of contaminants and naturally-occurring organic compounds.

5.4. Biodegradation of Specific Site Contaminants Under Aerobic Conditions

Aerobic respirometry was used to determine sediment-based bioremediation of site contaminants in oxygenated conditions. Exponential oxygen exertion was observed in all sediments with acetone, phenol, benzene, chlorobenzene, and 1,4-dichlorobenzene. Sediments showed exponential increases with acetone at 60-100 hours of experimental run time in the respirometer reactor (Figure 4.4.1.). Once initiated, exponential oxygen exertion with acetone occurred at rates of 0.048 hr^{-1} to 0.16 hr^{-1} (Tables 4.4.1 and 4.4.2.). Sediments responded with shorter experimental run times before oxygen exertion and with faster rates, as compared to acetone, when phenol was utilized as a substrate. Exponential growth on phenol occurred at 20 hours and was complete before 40 hours (Figure 4.4.3.). Utilization rates for phenol ranged from 0.1385 hr^{-1} to 0.287 hr^{-1}, averaging 0.22 hr^{-1}.

For benzene, exponential-growth, starting times ranged from 50 to 110 hours (Figure 4.4.5.). Degradation rates of benzene in the sediments ranged from 0.06974 to 0.15740 hr^{-1}. Chlorobenzene responded with less experimental run time before exponential growth, and the results show initiation of exponential growth ranging from 30 hours to 70 hours (Figure 4.4.7.). Kinetic rate data for chlorobenzene were also faster than benzene with a range from 0.08373 hr^{-1} to 0.21140 hr^{-1}.

Sediments from SITE-03 responded to 1,4-dichlorobenzene after shorter incubation times when compared to SED-01 and SED-08. SED-03 and SED-03* responded exponentially at 60-80 hours, while SED-01 and SED-08 responded after 150-170 hours of incubation (Figure 4.4.9.). Rates for 1,4-dichlorobenzene utilization were

lower than other specific contaminant rates. 1,4-dichlorobenzene rates ranged from 0.01194 hr^{-1} to 0.08612 hr^{-1}.

No net oxygen exertion was observed over a 7-day incubation period with TCE as substrate. Thus, intrinsic bioremediation did not appear to be feasible for TCE under aerobic conditions. Further focused investigation of TCE in aerobic systems indicated that TCE was inhibitory to the degradation of natural organic matter at TCE concentrations of 10-34 mg/L. The presence of TCE did not inhibit degradation of mixtures of easily-degraded compounds or mixtures of specific site contaminants, although subsequent degradation of natural organic matter was inhibited at 34 mg/L of TCE.

5.5. Biodegradation of Specific Site Contaminants Under Anaerobic Conditions

Biological conversion of contaminant carbon to carbon dioxide is a standard means to access biodegradability in the laboratory. Carbon dioxide production is a widely used measurement of mineralization or the complete oxidation of parent compounds. Under ideal conditions, mineralization is proportional to the amount of contaminant present and can be used to establish a mass balance for biodegradation. In this study, measurements of [^{14}C]-CO_2 produced from utilizing [^{14}C]-labeled organic contaminant by indigenous microorganisms was used to measure respiratory processes.

For site sediments with addition of radiolabeled compounds (acetone, benzene, chlorobenzene and 1,4-dichlorobenzene), only acetone was mineralized to $^{14}CO_2$ under anaerobic conditions. No CO_2-mineralization of benzene, chlorobenzene and 1,4-dichlorobenzene was observed.

Mineralization of acetone is a direct evidence of biodegradation. Acetone is decomposed by bacteria for metabolism and energy with a fairly rapid rate. Ten days of acclimation period (i.e., no degradation of chemicals) was observed on methane production and $^{14}CO_2$ production.

No CO_2-mineralization was observed for benzene and chlorobenzene and they are believed to be recalcitrant under methanogenic conditions. 1,4-Dichlorobenzene was not mineralized or degraded in the studies using labeled and unlabeled compounds. Degradation of trichloroethylene (TCE) was observed by a rapid disappearance of unlabeled TCE. TCE (~10 mg/L) was degraded in 30 days under methanogenic conditions.

Based on the current observations, it can be concluded that if the site is contaminated with water-immisible hydrocarbons, yet microorganisms generally will reside in the aqueous phase. For effective biodegradation to occur, it is therefore essential that the contaminant substrate be "bioavailable" to the degrading microbial communities. Many microorganisms possess an ability to overcome partitioning effects

and utilize water-insoluble substrates (i.e., 1,4-dichlorobenzene). In some cases, the production of extracellular, surface active agents are produced that may solubilize hydrocarbons into the aqueous phase (Miller, 1994). In other cases, hydrophobic cell walls may actually partition with hydrocarbons present in the soil or attach to water-hydrophobe interface. The mechanisms by hydrocarbon transport into, and assimilation by, microorganisms is not entirely understood (Bossert and Compeau, 1995).

Moreover, the chemical structure of a contaminant has both direct and indirect impacts on how well the substrate will be metabolized, i.e., biodegraded. First, metabolic or physiological constraints including nutrient or metabolic limitation and bioavailability of the contaminant substrate by microorganisms will directly impact how readily substrate can be degraded. Second, type and size of chemical structure may directly affect biodegradation by altering the bioavailability of the contamination to the biodegrading microorganisms. Biodegradability of hydrocarbons in soils has been demonstrated to correlate to their water solubility, which are generally inversely proportional to their respective molecular weight. Other structural attributes, such as degree of unsaturation, can affect water solubility and ultimately uptake and availability to the degrading microorganisms (Bossert and Compeau, 1995).

The effect of hydrocarbon contamination on microbial populations in soil therefore varies considerably, depending on the type, amount, and age of the contaminant, as well as the prevailing environment conditions. Unlike surface bodies of water or groundwater, which will diffuse the effects of contamination through dilution and migration, the contamination in soils will remain localized, potentially exerting pronounced effects on the immediate soil microcosms. However, because of physical matrix and chemistry of soil, most soils are actually good mitigates for toxic effects. By (ionic or covalent) binding , and/or sorption onto the soil organic materials which provide a solid, physical support as well to help protect and stabilize microorganisms and their cellular components, contamination quickly becomes immobilized and less bioavailable. Moreover, the solid soil structure aids in maintaining cell membrane integrity in the presence of contaminants exhibiting solvation effects (Bossert and Compeau, 1995).

Sorption described the partitioning of contaminants between aqueous phase and the solid aquifer matrix is not directly examined in the laboratory. However, quantity of sorbed contaminant in each bioreactor can be determine by direct counting of radiolabeled material on sediment or recovered throughout solid-liquid extraction from sediment. Sorption processes as we believed tend to reduce the dissolved contaminant concentrations and limits the migration of the aqueous phase plume, however, they do not result in a loss of contaminated mass from the aquifer.

6. References

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